The Effects of Simulated Urban Pollution on Trees and Shrubs

Catherine Shields

A thesis submitted for the degree of
Philosophiae Doctor

School of Biology
University of Newcastle upon Tyne

November 2003

The Effects of Simulated Urban Pollution on Trees and Shrubs

Catherine Shields

A thesis submitted for the degree of
Philosophiae Doctor

School of Biology
University of Newcastle upon Tyne

November 2003

I hereby declare that this thesis is based on work conducted by myself, and that the work has not been accepted in substance for any other degree. All references and ideas to work of other researchers has been specifically acknowledged.

C Shields

Abstract

In urban situations, plants are exposed to atmospheric pollutants derived from vehicle emissions in conjunction with hostile conditions such as low water availability, elevated temperatures and poor, compacted soils. This study used an experimental fumigation system for exposing trees and shrubs to simulated polluted urban atmospheres under near-natural environmental conditions. It also examined the interactions in plant responses to pollution and other stresses commonly encountered by plants in the urban environment.

Initial screening studies of 19 broad-leaved tree and shrub species revealed variation between species in responses to urban pollution mixtures. Stomatal conductance was generally suppressed in responding species. In further studies, the pollution regime was found to afford protection against drought by delaying wilting in *Ligustrum ovalifolium*. However, its effect of decreasing the root : shoot ratio in this species might impair drought resistance in the longer-term. In several species, urban pollution accelerated the onset of leaf senescence, which might be expected to have implications for long-term growth and survival in perennial species. In *Quercus robur*, there was a marked detrimental effect of exhaust gas pollution on surface waxes of the leaf cuticle, with associated changes in the water-repellency of leaves. A study of the common fungal disease of sycamore, tarspot, showed infection to be drastically impaired by urban pollution at the concentrations used in this fumigation.

Overall, there were surprisingly subtle effects on physiology in these species of a quite severe pollution treatment that represented the atmosphere of a polluted urban canyon. It may be that slow-growing perennial tree and shrub species such as those studied here are highly pollution-tolerant, or perhaps effects of pollution become apparent only over several growing seasons.

Acknowledgements

I am grateful to my supervisor, Alan Davison, for his continual support and enthusiasm throughout this project. Many thanks to the members of the Newcastle Air Pollution Laboratory, technical staff in the School of Biology at Newcastle University and the staff at CEH, Bangor who all offered advice and encouragement. Thanks also to the other members of the Urban Regeneration and the Environment programme for sharing their expertise.

Neil Cape and Jillian Binnie of CEH, Edinburgh performed VOC and HONO analyses. Particulate measurements were carried out by Keely Bignal of Bradford University. Analysis of foliar nitrogen concentration was carried out by Edward Okello of the of the Analytical Services Consultancy, School of Biology, University of Newcastle. Gillian Taylor of the Biomedical Mass Spectrometry Unit, University of Newcastle, ran the carbon isotope analyses.

Thankyou to my Mum and Dad, my brothers and sisters, and my friends, who all supported and encouraged me so much.

This work was funded by NERC (URGENT).

Table of Contents

Abstract i

Acknowledgements ii

Contents iii

Abbreviations x

1 General Introduction 1

1.1 The history of urban air pollution 1

1.2 Modern urban pollution climates 1

1.3 Formation, concentrations and characteristics of urban pollutants 1

1.3.1 Oxides of nitrogen (NO_x) 1

1.3.2 Carbon dioxide 6

1.3.3 Carbon monoxide 6

1.3.4 Particulates 7

1.3.5 Volatile organic compounds (VOCs) 8

1.3.6 Ozone 10

1.3.7 Nitrous acid (HONO) 14

1.4 Studies of the effects of urban pollution on plants 14

1.5 Uptake and fates of pollutants in plants 16

1.6 Effects of urban pollutants on plant physiology 19

1.6.1 Photosynthesis 19

1.6.2 Respiration 19

1.6.3 Stomatal response 19

1.6.4 Growth 20

1.6.5 Surface characteristics 21

1.7 Characteristics of urban habitats 22

1.7.1 Climatic features 22

1.7.2 Soils 23

1.8 Interactions of urban pollution with other stresses 24
1.8.1 Insect herbivory 24
1.8.2 Fungal pathogens 25
1.8.3 Drought 25
1.8.4 Frost 25
1.9 Critical levels for NO_x 26
1.10 The uses of vegetation in cities 26
1.11 Aims of this project 27

1 Materials and Methods 28
2.1 Exposure system 28
2.1.1 Solardome glasshouses 28
2.1.2 Pollution production and delivery 28
2.1.3 Control of pollutant concentrations 30
2.1.4 Comments on changes to the exposure system 31
2.1.5 Environmental conditions 31
2.1.6 Methods of pollution monitoring in the Solardomes 32
2.1.7 Results of pollution monitoring in the Solardomes 34
2.2 Plant Measurements 43
2.2.1 Growing conditions 43
2.2.2 Stomatal conductance 43
2.2.3 Gas exchange and photosynthesis 43
2.2.4 Chlorophyll fluorescence 45
2.2.5 Droplet contact angle measurements 45
2.2.6 Leaf drying rates 46
2.2.7 Water status 47
2.2.8 Stable carbon isotope discrimination 48
2.2.9 Nitrate reductase activity 49
2.2.10 Measurement of NO_2 concentrations 50
2.2.11 Overview of plant measurements 51

3 Initial screening experiment and selection of study species 53

3.1 Introduction 53

3.2 Materials and Methods 53

3.2.1 Plant material 53

3.2.2 Stomatal conductance 54

3.2.3 Contact angle measurements 54

3.2.4 Leaf drying rates 55

3.2.5 Growth 55

3.2.6 Statistical analysis 55

3.3 Results 55

3.3.1 Growth 58

3.3.2 Stomatal conductance 58

3.3.3 Contact angle measurements 58

3.3.4 Leaf drying rates 65

3.4 Discussion 67

4 The effects of urban pollution mixtures on response to water stress 69

4.1 Introduction 69

4.1.1 Influence of air pollutants on gas exchange and photosynthesis 69

4.1.2 Interactions between air pollutants and water stress 71

4.1.3 Aims of this study 73

4.2 Materials and Methods 73

4.2.1 Plant material 73

4.2.2 Drought regime 73

4.2.3 Stomatal conductance 73

4.2.4 Water status 74

4.2.5 Soil moisture 74

4.2.6 Stable carbon isotope discrimination 74
4.2.7 Growth 74
4.2.8 Biomass 74
4.2.9 Statistical analysis 75
4.3 Results 75
4.3.1 Stomatal conductance 75
4.3.2 Water status and soil moisture 80
4.3.3 Stable carbon isotope discrimination 85
4.3.4 Growth 86
4.3.5 Biomass 89
4.4 Discussion 93

5 The influence of nitrogen deposition on plant response to urban pollution mixtures 99
5.1 Introduction 99
5.1.1 Effects of enhanced nitrogen deposition on ecosystems 99
5.1.2 Effects of enhanced nitrogen deposition on plants 100
5.1.3 Aims of this study 103
5.2 Materials and Methods 103
5.2.1 Plant material 103
5.2.2 Addition of nitrogen 103
5.2.3 Stomatal conductance 103
5.2.4 Gas exchange and Photosynthesis 104
5.2.5 Chlorophyll fluorescence 104
5.2.6 Nitrate reductase (NR) activity 104
5.2.7 Growth 104
5.2.8 Biomass 104
5.2.9 Analysis of foliar nitrogen concentration 104
5.2.10 Leaf retention near the end of the growing season 105

5.2.11 Senescence in *Cornus sanguinea* determined by leaf colour 105
5.2.12 Senescence in *Cornus sanguinea* determined by chlorophyll fluorescence 105
5.2.13 Statistical analysis 105
5.3 Results 106
5.3.1 Stomatal conductance 106
5.3.2 Gas exchange and photosynthesis 106
5.3.3 Chlorophyll fluorescence 110
5.3.4 Nitrate reductase activity 112
5.3.5 Foliar nitrogen concentration 114
5.3.6 Growth 115
5.3.6 Biomass 116
5.3.7 Leaf retention near the end of the growing season 119
5.3.8 Senescence in *Cornus sanguinea* determined by leaf colour 120
5.3.9 Senescence in *Cornus sanguinea* determined by chlorophyll fluorescence 120
5.4 Discussion 121

6 Effects of urban pollution on leaf surface characteristics 128
6.1 Introduction 128
6.1.1 Structure, function and composition of epicuticular waxes 128
6.1.2 Influence of environmental factors on epicuticular waxes 129
6.1.3 Studies of epicuticular waxes 129
6.1.4 Leaf surface wettability 130
6.1.5 Effects of air pollutants 131
6.1.6 Aims of this study 134

6.2 Materials and Methods 134
6.2.1 Plant material 134
6.2.2 Droplet contact angle measurements 134
6.2.3 SEM observations 135
6.2.4 Statistical Analysis 137
6.3 Results 138
6.3.1 Droplet contact angle measurements 138
6.3.2 SEM observations on *Quercus robur* plants from the Solardome experiment 141
6.3.3 Field survey of *Quercus robur* trees 143
6.4 Discussion 145

7 The effects of urban pollution on infection by tarspot disease 148
7.1 Introduction 148
7.1.1 Life cycle of *Rhytisma acerinium* 148
7.1.2 *Rhytisma* and SO_2 pollution 149
7.1.3 *Rhytisma* and current urban pollution climates 150
7.1.4 Aims of this study 151
7.2 Materials and Methods 151
7.2.1 The tar spot index (TSI) 151
7.2.2 Solardome studies 152
7.2.3 Field surveys 153
7.2.4 Statistical Analysis 153
7.3 Results 154
7.3.1 Solardome studies 154
7.3.2 Field surveys 155
7.4 Discussion 158

8 **General Discussion** 161

8.1 The successes and limitations of the Solardome exposure system 161

8.2 Effects of exhaust gas pollution on plant growth, biomass partitioning and leaf senescence 164

8.3 Effects of exhaust gas pollution on gas exchange and photosynthesis 165

8.4 The influence of exhaust gas pollution on surface characteristics 167

8.5 The influence of exhaust gas pollution in conjunction with plant stress 167

8.6 Identification of species useful for urban planting 169

8.7 Suggestions for future study 170

References 172

Appendices 194

Abbreviations

A	CO_2 assimilation rate
A_{sat}	light-saturated CO_2 assimilation rate
ANOVA	analysis of variance
CFA	Charcoal/Purafil® filtered air
CEH	Centre for Ecology and Hydrology
CO_2	carbon dioxide
DoE	Department of the Environment
Fv/Fm	maximal quantum efficiency of Photosystem II
G_s	stomatal conductance
G_{ssat}	stomatal conductance at saturated light
HCHO	formaldehyde
HCO_3^-	bicarbonate
HNO_3	nitric acid
HONO	nitrous acid
HO_2	hydroperoxy radical
NEGTAP	National Expert Group on Transboundary Air Pollution
NH_3	ammonia
NH_4^+	ammonium ion
NH_4NO_3	ammonium nitrate
NO	nitrogen monoxide
NO_2	nitrogen dioxide
NO_2^-	nitrite
NO_3^-	nitrate
NR	nitrate reductase
NiR	nitrite reductase
O	elemental oxygen
O_3	ozone
OH	hydroxyl radical

PPFD	photosynthetic photon flux density
RH	hydrocarbons
RO_2	peroxy radical
RO	reactive oxygen species
R:S	root : shoot weight ratio
RUBISCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
SO_2	sulphur dioxide
UN/ECE	United Nations Economic Development Commission for Europe
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation

Chapter 1: General Introduction

1.1 The history of urban air pollution

Prior to the 1960's in the UK, domestic burning of coal in towns and cities contributed to the formation of smoke fogs (smogs) of SO_2 and smoke particles. In London in the winter of 1952, a heavy 4-day smog together with severe weather conditions was responsible for an estimated 4000 excess deaths. This led to the introduction of the Clean Air Act, 1956 to control the burning of smoky fuels in cities (DoE, 1995). There was a move from coal to natural gas for domestic heating, and power generation was centralized. Urban air quality improved dramatically. However, with increasing traffic volumes over the following decades, motor vehicles have become the most important source of urban air pollution.

1.2 Modern urban pollution climates

The major constituents of traffic pollution are NO_x, CO, CO_2, VOCs and particulates, with O_3 and peroxyacetyl nitrate (PAN) formed as secondary pollutants. Urban environments always contain complex mixtures of these pollutants and they are often present in high concentrations. Urban pollution causes an estimated 0.5 – 1 million premature deaths per year in developing countries around the world (Kojima and Lovei, 2001), and may still be causing health effects even at the lower concentrations found in developed countries.

1.3 Formation, concentrations and characteristics of urban pollutants

1.3.1 Oxides on nitrogen (NO_x)

Nitrogen monoxide (NO) is formed by the reaction of nitrogen and oxygen in combustion processes, with one atom of each gas combining. NO is the dominant component of exhaust gases. It can be rapidly oxidized into nitrogen dioxide (NO_2), the speed of the reaction depending on the availability of oxidizing agents (e.g. O_3). At the roadside, NO typically makes up around 80% of total NO_x (e.g. Wellburn, 1990), while background city air contains equal amounts of NO and

NO_2 (e.g. Nielsen *et al.*, 1995). Natural sources of NO_x include lightning, forest fires and the activity of certain soil bacteria. Fossil fuel burning is the largest source, especially by motor vehicles and power stations.

When NO from vehicle emissions is oxidised to form NO_2, the oxygen atoms often come from O_3 (Equation 1). Later, under the influence of sunlight, this NO_2 can lead to the photochemical formation of further O_3 (Figure 1.3). Alternatively, NO can be converted to NO_2 through oxidation by atmospheric O_2 (Equation 2). This is the more important mechanism in wintertime, during stagnant, cold weather. Under such conditions, several localized NO_2 pollution episodes have occurred in the UK. The most severe happened in London in December 1991, where an hourly average concentration of 423 ppb NO_2 was recorded (DoE, 1996).

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad \textbf{Equation 1}$$

$$2\,NO + O_2 \rightarrow 2\,NO_2 \qquad \textbf{Equation 2}$$

Once in the atmosphere, NO_2 can be converted to nitric acid and nitrates, which can be removed by rain through wet deposition. Nitrates may also remain in the air as very small particles (in the PM_{10} range) such as ammonium nitrate (DoE, 1996). Of a total UK nitrogen deposition of 380 kt-N ha^{-1} y^{-1}, oxidized nitrogen contributes 43% and reduced nitrogen 53%. Although actual *emissions* of oxidized nitrogen are greater than those of reduced N, oxidized nitrogen is transported further from its source, with 85% being deposited outside the UK (NEGTAP, 2001). A map of total nitrogen deposition for the UK in 1997 is given in Figure 1.1.

Over 1 million tonnes of NO_2 are produced per year in the UK by motor vehicles and half a million tonnes by non-nuclear power stations (DoE, 1996). Major road links produce 33% of NO_x emissions in the UK. On NO_x emission maps,

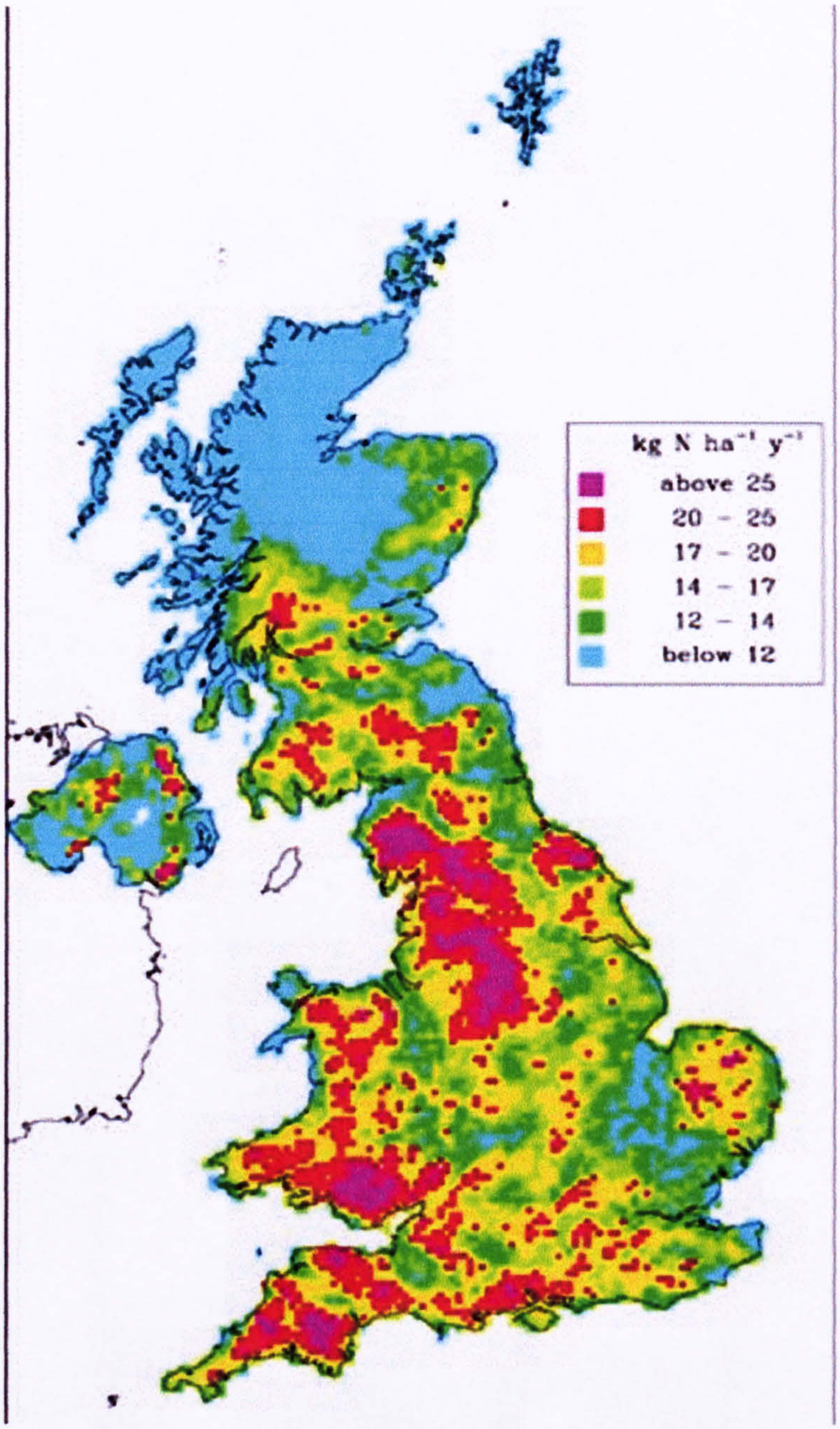


Figure 1.1 Total deposition of nitrogen in the UK in 1997. Map courtesy of CEH.

motorways and urban areas can be seen to stand out. Large urban areas have annual average concentrations of around 20-30 ppb, compared with values of 10 – 15 ppb in extensive rural areas of the Midlands and South East England. The cleanest areas of the UK (the Highlands of Scotland and the West coast) have annual average concentrations below 1 ppb (NEGTAP, 2001). From 1995 data, maximal hourly average concentrations above 100 ppb were recorded in many major cities. However, the number of hours where the DoE panel's recommended standard of 150 ppb was exceeded were few (DoE, 1996). Ground-level concentrations in urban areas are determined more by vehicle emissions than by power stations, as tall stacks efficiently disperse pollutants.

Between 1970 and 1998, there was an overall reduction of 30% in NO_x emissions in the UK (Goodwin et al., 2000), although this was not a constant decline (Figure 1.2). The growth in road traffic from 1984 onwards was reflected in a marked increase in NO_x emissions, with a peak in 1989. After this, emissions decreased by 38% due to a reduction from power stations (of 53%) and from road transport (of 42%). Despite continuing increases in traffic volumes, the growth of catalytic converters has more than counteracted this effect. More catalytic converters in vehicles are predicted to further reduce NO_x emissions by 74% between 1998 and 2010 (NEGTAP, 2001).

NO does not affect human health at the concentrations found in ambient air. It has been known for a long time that at very high concentrations, NO_2 has serious deleterious effects. Recent studies have suggested it could also have subtle, long-term effects on health at lower concentrations that may be encountered in the city atmosphere. People suffering from respiratory illnesses such as asthma are more sensitive to the effects of the gas than healthy people. The DOE Expert Panel recommend an Air Quality Standard of 150 ppb NO_2 measured as an hourly average, but state that a longer-term standard may be required. Epidemiological studies suggest that NO_2 may have adverse health effects at lower concentrations over longer periods of time (DoE, 1996).

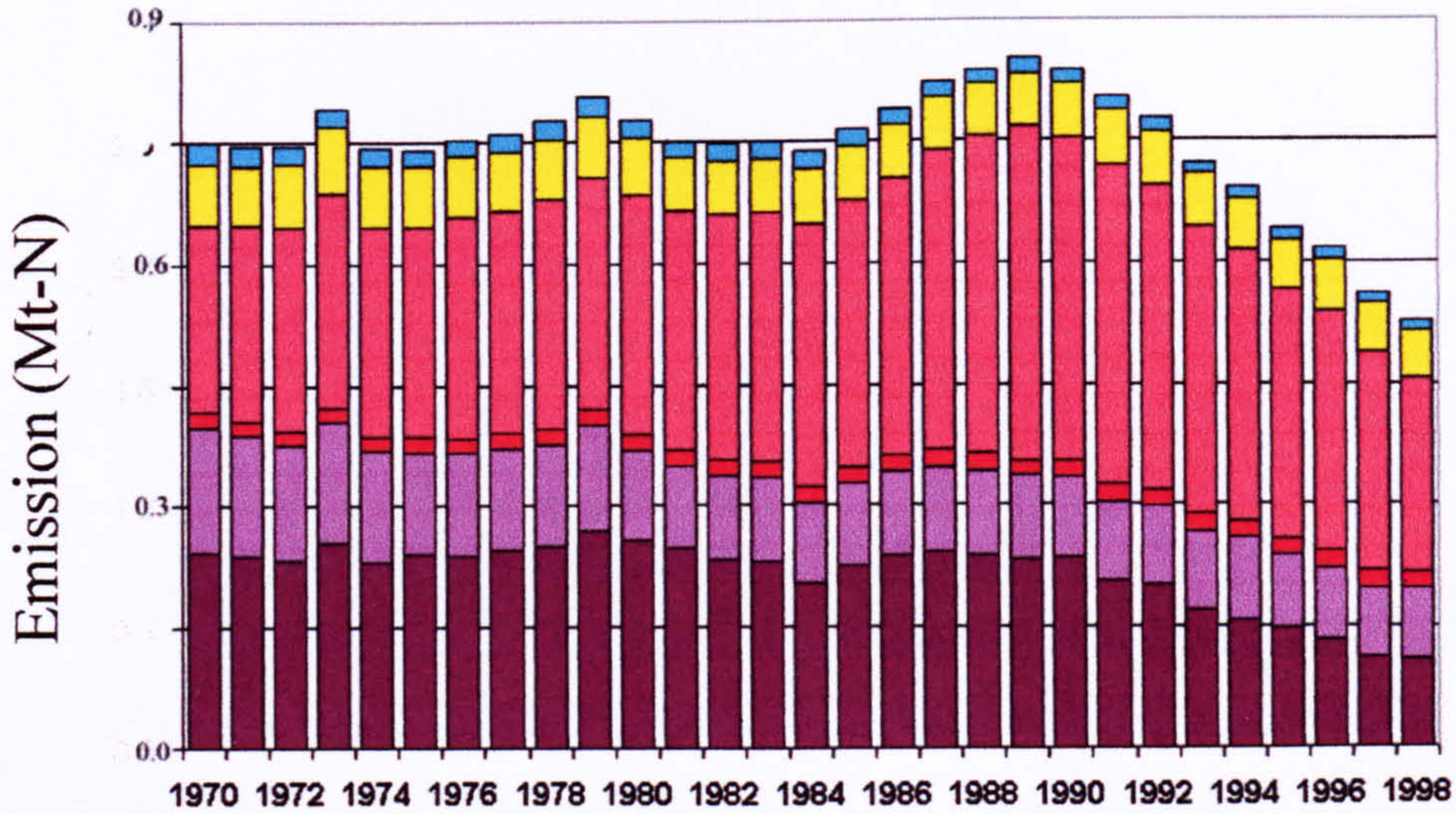


Figure 1.2 UK emissions of NO_x (Mt-N) between 1970 and 1998. Adapted from Goodwin *et al*. (2000).

1.3.2 Carbon dioxide (CO_2)

Combustion of any organic substance produces CO_2. Concentrations in urban areas show a diurnal cycle, with peaks at rush hours, when they can reach 24 ppm higher than surrounding rural areas (e.g. Nemitz *et al.*, 2002; Day *et al.*, 2002). CO_2 does not directly impair human health at concentrations currently found in cities (USEPA website). Concerns over emissions of CO_2 are related to its contribution to the global greenhouse effect.

1.3.3 Carbon monoxide (CO)

Complete combustion of organic substances generates CO_2, but where there is insufficient O_2, CO instead is formed. When a vehicle is moving slowly, or its engine is cold or badly tuned, more CO is produced compared with when there is just enough air to oxidise the fuel's carbon. Emission maps show high concentrations in areas where traffic volumes are high, and emissions are particularly high in city centres where traffic speeds are slow (NEGTAP, 2001). Vehicle-derived CO is dispersed away from roads and, over months, is broken down by photochemical reactions (contributing to the formation of tropospheric O_3). As with NO_2, cold, still weather conditions can hinder its dispersal.

In the UK in 1998, an estimated 5 million tonnes of CO were released into the atmosphere. Road transport was the source of 73% of emissions (NEGTAP, 2001). Between 1970 and 1990, there was an increase in emissions of 50% as traffic volumes grew (DoE, 1994a). With the increase in catalytic converters, emissions have declined by 31% between 1990 and 1998. This trend is expected to continue as new cars with improved engine design are introduced. By 2020 CO emissions are predicted to decrease by a further 41% compared with 1998 levels (NEGTAP, 2001).

1991 data give annual average concentrations of around 1 ppm for major cities other than London. Near London's busiest roads, annual averages of up to 3 ppm were recorded. Maximum 1-hour averages were in the teens for many major

cities. The DoE panel's recommended (1994a) 1-hour average of 25ppm was not exceeded, but their 8-hour average standard of 10 ppm was occasionally exceeded in the largest cities.

The threat posed to health by CO is a reduction in the oxygen-carrying capacity of the blood, by increasing the level of caboxyhaemoglobin. In healthy people, there is no noticeable effect on exercise performance until relatively high levels of caboxyhaemoglobin are reached. People suffering from angina and disease of the coronary arteries are most susceptible to exposure (DoE, 1994a).

1.3.4 Particulates

With respect to effects on human health, the most important particulates are those in the PM_{10} range (less than 10 μm in diameter), those most likely to be deposited in the lung. Within the PM_{10} range, particulates can be subdivided into three size ranges. Those in the nucleation mode (<0.2 μm) are formed by condensation of hot vapors, for example from vehicle exhausts, and by conversion of gases to particles in atmosphere. They rapidly aggregate to form larger particles. These are accumulation mode particles, between 0.2 and 2 μm diameter, which have longer existences and can remain in the air for 7 – 30 days. Those particles above 2 μm diameter are in the coarse mode, composed largely of minerals derived from soil, sea spray and industrial processes. Large, heavy particles remain suspended in the air for a short time (DoE, 1995).

Particulates are of very different chemical composition depending on their sources. For example, near the coast, salt can be the major contributor to suspended particulate matter. In urban areas, the greatest source of particulate emissions is vehicle pollution, contributing an estimated 86% of primary PM_{10} in Greater London in 1990. For the UK as a whole in the same year, around 24% of PM_{10} originated from road traffic (DoE, 1995). Annual average concentrations of PM_{10} taken in 1994 in major UK cities are mostly in the mid-twenties $\mu g\ m^{-3}$.

Maximum hourly concentrations were mostly in the range of 200 – 320 μg m^{-3} (DoE, 1995).

Road vehicles also contribute to the formation of secondary particles, when nitrogen oxides are neutralised by atmospheric ammonia derived from agricultural sources to form ammonium nitrate. These secondary particles are widely distributed across the country, some being exported outside the UK (DoE, 1995).

Of particles in the PM_{10} range, those < 0.1 μm diameter have the greatest probability of reaching the alveoli of the lung. Almost all particles > 7 μm are deposited instead in the nose and throat (DoE, 1995). All evidence for particulates affecting health comes from population studies looking at the relationship between ill health and pollution episodes or background pollution levels. As with most other pollutants, particulates are likely to affect people made susceptible by pre-existing heart and lung diseases. Whether long-term exposure to particulate pollution could contribute to the development of such illnesses is still uncertain.

The DoE panel (1995) have recommended a standard of 50 μg/m3 for PM_{10}, measured as a 24-h running average. This is believed to be a safe level of exposure for the very large majority of the population. In the UK, this concentration has regularly been exceeded in most urban areas, especially in wintertime.

1.3.5 Volatile organic compounds (VOCs)

VOCs are found at ground level in all urban centres. A broad definition encompasses any carbon-containing compounds found in the atmosphere (excluding elemental C, CO and CO_2) (Derwent, 1995). This includes those in the gaseous phase, and those semi-volatile organic compounds that are adsorbed

onto the surfaces of suspended particulate matter. VOCs are very diverse, comprising many hundreds of compounds which are mainly, but not exclusively, hydrocarbons (Harrison, 1996).

Natural sources of VOCs include plants and trees, forest fires and anaerobic processes in bogs and marshes (Derwent, 1995). Recent estimates show around 40% of VOCs in Europe to be from biogenic emissions (Simpson *et al.*, 1999). The major sources from human activity are vehicle exhaust, solvent use in industrial processes, evaporation of petrol from cars and extraction and distillation of fossil fuels. Vehicle exhaust contributed 33.7% of anthropogenic hydrocarbon emissions in the UK in 1993 (Harrison, 1996). In the London area, up to 53% of all anthropogenic VOCs are generated by vehicle emissions (Salway *et al*, 1996 in Binnie *et al.*, 2002).

Twenty-six individual hydrocarbons are monitored hourly by the UK Hydrocarbon Network in 11 urban sites and one rural site. Let us take three of these compounds as examples. From 1998-2000 data, benzene, toluene and ethylene were found at similar average "background" concentrations at all the urban sites (around 2-3 ng l^{-1} for benzene and ethylene and around 5-7 ng l^{-1} for toluene). At a kerbside site on a busy London street, averages were much larger, and at a rural site they were around half those of urban concentrations. Maximal hourly concentrations were up to 100 times greater than averages (Derwent, 1995).

An important class of VOC are polynuclear aromatic hydrocarbons (PAH), consisting of two or more fused aromatic rings. They are formed in incomplete combustion or high-temperature pyrolytic process involving fossil fuels. Vehicle exhaust is the major source in urban atmospheres, particularly from heavy diesel vehicles and cars with defective catalysts (Gerdol *et al.*, 2002). PAH are generated in gaseous phase and are then massively adsorbed onto pre-existing particles, mainly those below 2 μm diameter, and in this form can be transported

long distances (Gerdol *et al.*, 2002). Several are carcinogenic, for example benz[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene (Alfiani *et al.*, 2001). Other recognised "air toxics" with potential carcinogenic effects are benzene and 1,3-butadiene (Derwent, 1995). Benzene and 1,3-butadiene have DoE recommended air quality standards of 5 ppb and 1 ppb respectively, measured as a rolling annual average. Other VOCs contribute to the formation of potentially harmful secondary pollutants such as O_3 and peroxyacetyl nitrate (PAN) through reactions with NO_x (Murlis, 1995).

1.3.6 Ozone

Ozone occurring naturally in the stratosphere is formed by the action of the sun's UV light on oxygen molecules. Natural periodic intrusions of air from the stratosphere to the troposphere bring in some O_3. This tropopause folding contributes to the global average background concentration of 20-30 ppb (NEGTAP, 2001). Elevated ozone levels in the lower atmosphere are largely the result of human activity. Ozone is not produced as a primary pollutant in appreciable amounts but is generated from photochemical reactions between NO_x and hydrocarbons (Figure 1.3). A range of molecules undergo photolysis in the presence of solar ultraviolet radiation to form hydrogen-containing free radicals (HO_2 and OH; hydroperoxy radical and hydroxyl radical, respectively). In atmospheres containing >30 ppt NO_2 these free radicals catalyse the oxidation of VOCs, e.g. hydrocarbons (RH), producing O_3 as a by-product. OH reacts with CO or hydrocarbons to form peroxy radicals (RO_2), which in turn oxidize NO to NO_2, forming O_3 (following the photolysis of NO_2 to NO and O). The conversion of RO radicals to HO_2 allows the formation of further OH (PORG, 1997; Fowler *et al.*, 1999). Formaldehyde (HCHO) is emitted from vehicle exhaust, and so is present at elevated concentrations in urban atmospheres. It contributes to free radical formation, and plays an important part in initiating O_3 production (PORG, 1997). Nitrous acid (HONO), generated in urban areas during the night, is a major source of OH radicals in the early morning (Figure 1.4).

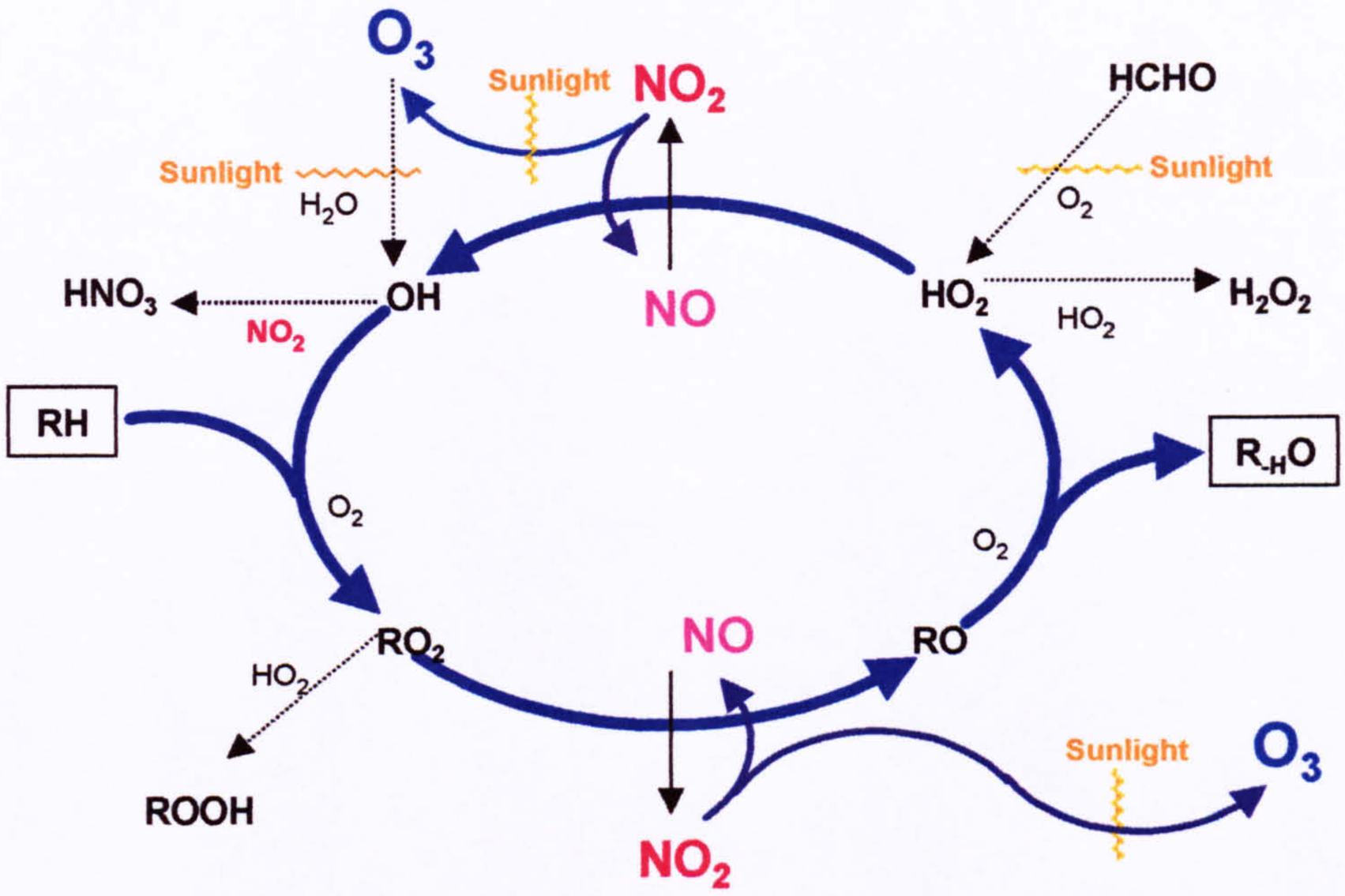


Figure 1.3 Schematic diagram showing the reactions involved in the formation and destruction of ozone. A range of molecules undergo photolysis in the presence of solar ultraviolet radiation to form hydrogen-containing free radicals (HO_2 and OH) in atmospheres containing >30 ppt NO_2 these free radicals catalyze the oxidation of VOCs (e.g. hydrocarbons, RH), producing O_3 as a by-product. Adapted from PORG (1997).

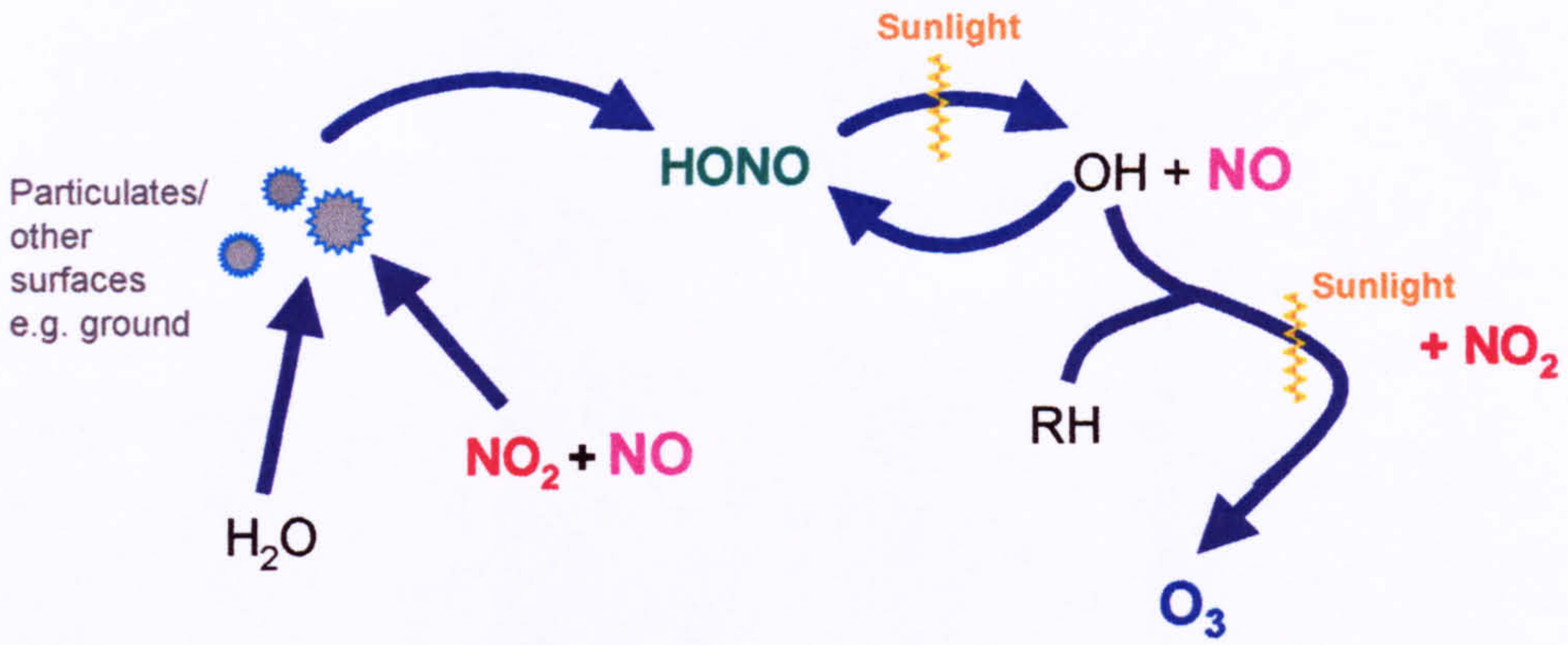


Figure 1.4 HONO, formed mainly by the reaction of NO_2 and NO on moist surfaces (e.g. particulates/ground), is a major source of the OH radical in the early morning. After building up over night, it undergoes photolysis in sunlight.

Once formed, O_3 can persist for several days, and is carried in the air long distances away from the sources of the primary pollutants that are its precursors. High NO concentrations in urban centres can react with and remove O_3 to form NO_2, so that O_3 concentrations are usually greater in suburban and rural areas than in cities. The annual average concentrations of O_3 in urban/industrial areas are around 10 ppb, while those in rural areas are around 25 ppb (NEGTAP, 2001). There are also seasonal and geographical variations. In the UK, the lowest concentrations are found in the northwest and the highest in the southeast during the summer. In the winter, overall concentrations are lower but the north-south gradient is reversed due to removal by NO in the dense urban regions of the south (NEGTAP, 2001).

High O_3 concentrations can occur in cities on hot, sunny, still days where there is high O_3 generation with poor dispersal. Under such conditions, photochemical

smogs have occurred in very polluted cities like Athens and Los Angeles, with concentrations sufficient for people to feel the irritant effects of the gas (DoE 1994b). In Europe, photochemical smogs do not occur, but elevated levels can still cause "episodes" where O_3 concentrations can exceed 100 ppb. In Britain such episodes occur only on 10-30 days in any one year (Harrison, 1996).

Ozone is known to have an irritant effect on delicate body tissues such as the eyes, nose and lungs, with inflammation of the surface of the lung's lining occurring when people are exposed to concentrations exceeding 100 ppb for several hours (DoE 1994b). At present, there is no evidence of long term effects on health at concentrations encountered in the UK (DoE 1994b). The DoE (1994b) recommend a standard of 50 ppb measured as a running 8-hour average, while the USEPA standard is 120 ppb, not to be exceeded as an hourly average more than once per year (Harrison, 1996). From 1996 data, both standards were exceeded several times a year in most parts of UK. In order to reach the proposed DoE standard, emissions of the O_3 precursors VOCs and NO_x would need to be cut by 75-80% and >95%, respectively, from 1996 levels (DoE 1994b).

1.3.7 Nitrous acid (HONO)

HONO is a little understood but important urban pollutant. Up to 1% of NO_x emitted from combustion processes is present as HONO (e.g. Kessler and Platt, 1984), making vehicle emissions an important source in areas of heavy traffic. Some HONO is formed by reactions between OH and NO, thus removing OH radicals from the atmosphere (Stuhl and Niki, 1972). However, the majority is formed heterogeneously in the presence of NO_x and water adsorbed onto particles, and on the ground and other surfaces (e.g. Harris *et al.*, 1982). These reactions are given in Figure 1.4.

There is a diurnal cycle in HONO concentrations, with a build-up overnight (up to concentrations of 2.9 ppb; e.g. Reisinger, 2000), and destruction by photolysis in the early morning hours. During this time HONO is a major source of OH

radicals, which catalyse O_3 formation (Figure 1.3). The effects of HONO on plants are not known, but based on its physico-chemical properties, Duyzer and Fowler (1994) suggest that it would be expected to be taken up by stomata. There are a few reports of HONO uptake by vegetation (Duyzer and Fowler, 1994).

1.4 Studies of the effects of urban pollution on plants

Some of the earliest studies of urban vegetation showed stunting of growth that was well correlated with estimated rates of SO_2 deposition (e.g. Cohen and Ruston, 1912, reviewed in Mansfield and Lucas, 1996). In the first half of the 20th century, apart from SO_2, the blocking out of light by smoke was likely to be involved in the observed stunting of plant growth. Problems associated with growing ornamental trees and shrubs in cities were recognised as early as 1928 (reviewed in Mansfield and Lucas, 1996). It might be expected that the trees and shrubs now found in urban areas are those that are pollution tolerant. Pollution has been present since at least the industrial revolution, and therefore it has been suggested that sensitive species/genotypes would have failed to survive in the city environment some time ago (Innes, 1988). However, the urban pollution climate has altered considerably in the last few decades, so that plants that were tolerant to the major pollutants of the past may not necessarily be resistant to modern pollution climates.

In modern cities, NO_2 is likely to be the major phytotoxic agent (Mansfield and Lucas, 1996). Consequently, NO_2 is the most-studied vehicle-derived pollutant. In fumigation studies, it has often been applied singly, or in combination with one or two other pollutants, usually SO_2 and/or O_3 (reviewed in Fangmeier *et al.*, 2002). These mixtures may no longer be relevant to urban situations since the pollution climate has altered in the last decade or so. Realistic pollution mixtures need to be used if interactions between pollutants are to be elucidated. Fumigation studies have also often involved exposing plants in short-term exposures to acute concentrations of pollutants (Fangmeier *et al.*, 2002).

Ideally, experimental exposure of plants to pollutants should be performed under "normal" growing conditions (Heagle and Philbeck, 1979). Chamberless outdoor fumigation systems give the most realistic growing environment, but do not allow the application of controlled pollutant concentrations, or for direct comparison with non-polluted control treatments (Treshaw and Bell, 2002). Open top chambers have been widely used, where polluted or filtered air is introduced through a perforated ring around the base of the chamber, giving an upward flow of air (Treshaw and Bell, 2002). Semi-natural conditions of light and moisture are maintained. However there can be vertical pollution gradients within the chamber due to incursion of air into the top. Also, differences in air movement may alter stomatal uptake compared with that found in the field (Unsworth, 1990). In air filtration systems (described in Treshaw and Bell, 2002), plants can be exposed to ambient polluted air and compared with plants in filtered air chambers. However, these systems are expensive and encounter many technical problems (Treshaw and Bell, 2002), and as with open-air exposures, pollutant concentrations cannot be controlled. Although closed chamber systems least resemble field conditions, environmental conditions of light, temperature and humidity and the availability of water and nutrients can be readily controlled, as can pollution concentrations (Unsworth, 1990).

1.5 Uptake and fates of pollutants in plants

The effects of pollutants on plant functioning depend on the physical and chemical features of the pollutants, such as their readiness to adsorb onto leaf surfaces, their solubility in cell sap and their ability to react with cellular components (Stulen et al., 1998). Some pollutants, such as the VOCs ethylene, isoprene, ethane, methanol, toluene and formaldehyde are also emitted by some plants (Cape, 2003). This could mean that since the biochemical pathways exist within the plant to regulate these compounds, plants might be able to cope well with elevated external concentrations. Conversely, plants might be expected to

be particularly sensitive to enhanced levels of VOCs that normally play a role as signaling molecules (Cape, 2003).

Features of the plant's physiology, for example stomatal conductance and metabolic rates, also determine the extent of pollutant uptake and effects (e.g. De Kok, 1990), and these can be altered by the presence of pollutants. In higher plants, cuticular uptake of NO_x is negligible, and pollutant entry is primarily through the stomata (e.g. Van Hove *et al.*, 1991). There is evidence that, at the roadside, turbulence from passing vehicles might increase the effective dose taken into plants, probably by reducing the boundary layer resistance at the leaf surface (e.g. Ashenden and Mansfield, 1977).

Due to its high solubility, NO_2 encounters little resistance inside the leaf, and its deposition velocity can be estimated from measurements of stomatal conductance and atmospheric NO_2 concentrations (Stulen et al., 1998; Fowler, 2002). Working with a range of tree species, Hanson and Lindberg (1991) showed stomata to be the only significant sink within the vegetation. The deposition velocity of NO_2 therefore follows a marked diurnal as well as a seasonal cycle, with maximal velocities in the daytime during the summer (Duyzer and Fowler, 1994). NO dissolves less readily than NO_2, and internal resistance to NO is high. The fate of NO after entry into the leaf is not certain, but some studies suggest it can be more toxic and inhibitory to plant growth compared with NO_2 (Darrall, 1989; Wellburn, 1990). It is a free radical (·N=O), capable of interfering with enzymes and reaction centres (Wellburn, 1990). Petrouleas and Diner (1990 in Mansfield and Lucas, 1996) found that NO may bind to a site on the PSII reaction centre, so competing directly with the normal substrates, HCO_3^- or CO_2.

In the apoplast, NO_2 dissolves to give species that form nitric acid (HNO_3) and nitrous acid (HONO) (Lee and Schwartz, 1981). At pH ranges found in cells, nitric acid ionises to form nitrate (NO_3^-). NO also forms nitrate and nitrite (NO_2^-)

ions, but at slower rates than NO_2 (Wellburn, 1990). Nitrate may be stored in vacuoles or reduced to nitrite in the cytoplasm by the action of the inducible enzyme, nitrate reductase (NR) (Stulen and Ter Steege, 1995). The resulting nitrite is transported into the chloroplast, where it is reduced to ammonium (NH_4^+) by nitrite reductase (NiR) and the GS/GOGAT cycle (Lea and Miflin, 1974). In order to prevent the build-up of potentially toxic ammonium ions, the plant quickly synthesizes them into amino acids (Rabe, 1990).

In some woody plant species, most nitrate reduction normally takes place in the roots so that nitrogen is already in reduced form when transported to the shoots. Indeed, the reaction pathways for reducing nitrogen are absent or poorly developed in the leaves of some species. They may be particularly sensitive to exposure to atmospheric NO_x, as their leaves lack the ability to detoxify the potentially harmful nitrite ions (Mansfield and Lucas, 1996).

Oxides of nitrogen are also deposited at the soil surface. Lindqvist *et al.* (1982, cited in Spencer and Port, 1988) found simulated road dirt to have a high potential for NO_2 absorption. In the soil, NO_2 is converted to nitrate and nitrite (Yoneyama, *et al.*, 1979), and can be taken up by plant roots (Yoneyama , *et al.*, 1980). Once inside the plant, this extra nitrogen can enter into the normal metabolic pathways described above, and become incorporated into amino acids.

Many species have been found to increase their amino acid content when exposed to NO_x (reviewed in Wellburn, 1990). In several radiotracer studies where plants have been treated with $^{15}NO_2$, certain amino acids became strongly labeled after only 20 minutes of exposure (reviewed in Wellburn, 1990). The individual amino acids whose concentrations are altered in response to NO_x vary between plant species, but several studies have agreed that in angiosperms, concentrations of asparagine and glutamine (e.g. Prasad and Rao, 1980; Ito *et al.*, 1984), proline and arginine (Ohlson et al., 1995) tend to increase.

VOCs can be accumulated in plant tissues (Brown *et al.*, 1999; Hiatt, 1999; Keymeulen *et al.*, 1993), but the extent of accumulation depends on plant species and the individual chemical compounds. Many early studies assumed VOCs to be inert once taken up by plants, and not actively metabolised or transported in the leaf (Cape, 2003). However, more recent studies have shown that at least for some aromatic hydrocarbons, concentrations measured in leaves are lower than would be expected from a simple equilibrium between concentrations in the air and those in the leaf (e.g. Binnie *et al.*, 2002). Benzene and toluene have been shown to be actively metabolised when present at high concentrations (reviewed in Cape, 2003). VOC deposition and absorption by plants varies greatly between different compounds, and also between plant species and plant organs. After being taken up by plants, subsequent release of benzene from denser plant parts such as fruits has been shown to be slower than for leaves (Collins *et al.*, 1998). Uptake of VOCs into plant tissues may be initially rapid until the sink becomes saturated, at which point an equilibrium is reached, as proposed by Collins *et al.* (2000) for benzene uptake by blackberry leaves. Vegetation may only be a temporary sink VOCs, with uptake at high air concentrations and release at low concentrations (Collins *et al.*, 2000).

1.6 Effects of urban pollutants on plant physiology

1.6.1 Photosynthesis

Few studies have looked at the effects of mixtures of urban pollutant gases on photosynthesis. Of the nitrogen oxides, NO appears to be more inhibitory to photosynthesis compared with NO_2 (Saxe, 1986). NO_x have been observed to cause both increases and reductions in net photosynthetic rates depending on plant species and growing conditions (Wellburn, 1990). Generally, NO_x appear to be less inhibitory compared with pollutants such as SO_2 and O_3. In a study of the effects of vehicle exhaust gases, Kammerbauer *et al.* (1987) found 31%

decreases in net assimilation rates of Norway spruce plants compared with controls.

1.6.2 Respiration

Exposure of plants to the air pollutants SO_2 and O_3 generally increases dark respiration rates (e.g. Black and Unsworth, 1979; Barnes, 1972). For NO_x, there are no consistent patterns of alterations in respiration. Both enhanced and suppressed respiration rates have been reported for different species (Darrall, 1989; Wellburn, 1990). Where respiration rates increase, this may aid in repair of pollution-induced damage (Darrall, 1989).

1.6.3 Stomatal response

Stomatal response to air pollutants is influenced by a variety of factors, such as pollution concentrations, plant species and growing conditions. Herbaceous plants have a well-defined stomatal closure response to CO_2, although trees seem to react differently. Some studies of woody species have found small or zero reductions in stomatal conductance, and there are some observations of increases in conductance (reviewed in Robinson *et al.*, 1998 and Mansfield, 1998). Different plant species have shown both increases and decreases in stomatal conductance in response to NO_2 (reviewed in Darrall, 1989). In a field study of exposure of Norway spruce at the roadside, Kammerbauer *et al.* (1987) observed decreased conductance compared with controls. Where Norway spruce were fumigated with mixtures of vehicle exhaust gases, there were no effects on conductance in the daytime, but enhanced conductance at night (Viskari *et al.*, 2000b).

A stomatal opening response is expected to increase the effective dose of pollution. It may be the result of damage to epidermal cells surrounding stomata, as observed in leaves exposed to mixtures of $SO_2 + NO_2$ (e.g. Neighbour *et al.*, 1988). Pollution-induced stomatal closure is probably a response to reduced photosynthetic rates (Robinson *et al.*, 1998). A side-effect is a reduction in the

uptake of pollutants, which is expected to protect tissues from injury (Darrall, 1989).

1.6.4 Growth

NO_2 has been found to cause both stimulation (e.g. Caporn et al., 2000) and inhibition (reviewed in Wellburn, 1990; Stulen *et al.*, 1998) of growth in different plant species. Where NO is also present, growth is often suppressed (Wellburn, 1990), possibly due to the accumulation of harmful nitrite ions in leaf tissues (Bell *et al.*, 1992). Where growth is increased, NO_x may appear to be initially beneficial to certain species as a source of growth-stimulating nutrients. However, in the longer term, it may have negative effects on physiology, since nitrogen-stimulated growth may exceed the availability of other essential nutrients, leading to secondary deficiencies (e.g. Miller and Miller, 1988). It may also affect resistance to environmental stresses and disrupt ecosystem function by altering the outcome of competitive interactions (e.g. Tamm, 1991; Aerts and Chapin, 2000). The ecosystems most sensitive to nitrogen additions are those that have naturally low nitrogen availability, for example heaths, moors and semi-natural grasslands (NEGTAP, 2000).

VOCs have the potential to alter plant growth (e.g. Cape *et al.*, 2003b). Uniquely among air pollutants, ethylene is a plant hormone and is emitted by plants as a non-specific response to stress (e.g. Cape, 2003). A concentration of only 20 nl l^{-1} ethylene has been shown to significantly increase pea epicotyl elongation (Goeschal and Kays, 1975). It also affected seed weight in canola, with increases in seed weight at low ethylene concentrations and reductions at high concentrations (Reid and Watson, 1985). Ethylene produced by plants plays a role in the normal progression of senescence of flowers (e.g. Porat and Halevy, 1993; Ichimura and Suto, 1998) and leaves (Davison, 1974). Isoprene, another VOC present in vehicle exhaust, accelerated the onset of flowering in several species of plants at concentrations of 50-150 nl l^{-1} (Terry *et al.*, 1995). A mixture

of six VOC species caused an acceleration in the formation of seedpods in *Lotus corniculatus* (Cape *et al.*, 2003b).

1.6.5 Surface characteristics

Accelerated structural erosion of leaf cuticular waxes has been reported in response to many pollutants, including particulates, ozone, NO_x and vehicle emissions (reviewed in Viskari *et al.*, 2000b). Many studies have looked at conifers, but changes in surface structures have also been observed in several broadleaved tree species (Huttunen, 1994). The mechanism(s) by which pollutants degrade plant cuticles are not certain. Air pollutants may interfere with the wax biosynthetic pathways (Kerfourn and Garrec, 1992) or undergo direct physicochemical reactions with wax crystals on the cuticle surface (Riederer and Schneider, 1989). Particulates might interact with waxes and cause direct physical abrasion (Cape, 1994).

In exhaust gas mixtures, organic hydrocarbons (e.g. benzene and xylene families) and NO_x are most likely to be responsible for wax damage (Sauter and Pambour, 1989). Some experiments with conifers have shown aromatic hydrocarbon "fumes" to produce similar wax degradation to those brought about after exposure to roadside atmospheres (Sauter and Pambor, 1989). NO_x are also candidates as agents damaging to cuticles. They are lipid soluble and may react directly with the cuticular waxes, but this may only be important at high concentrations (e.g. Lendzian and Kersteins, 1988, cited in Cape, 1994).

Damaged cuticles may have altered sorptive and transport properties, with increased cuticular water loss and enhanced permeability to gases (Lendzian and Kersteins, 1991). On erosion, epistomatal wax crystal structures of conifers have been found to flatten and fuse together, sometimes completely blocking the stomatal chamber (reviewed in Viskari *et al.*, 2000b). Pollution-induced injury to cuticles also has the potential to influence attack by pests and diseases. Surface lipids are known to influence insect attachment, movement, oviposition and

feeding (Eigenbrode and Espelie, 1995), as well as attachment and hypahl growth of fungal pathogens (Turunen and Huttunen, 1990).

1.7 Characteristics of urban habitats

1.7.1 Climatic features

Urban environments can be hostile, exacerbating the effects of air pollution on vegetation (Madders and Lawrence, 1981). Table 1.1 summarizes some of the differences between the climate of an urban centre compared with surrounding rural areas. There is a temperature difference between urban and rural areas, the "heat island" effect, with parts of London reaching temperatures as much as 6° C warmer than surrounding rural areas (Chandler, 1965). Materials in the city have a high thermal capacity, which allows the temperature to build during the day. This temperature effect is aided by the burning of fuel. In London, this leads to an extension of three months in the active growing season for plants, and a delay of ten weeks in the onset of winter frosts (Gilbert, 1989).

The frictional drag of buildings on the air moving past them reduces wind speed, although tall buildings can create local eddies at ground level (Gilbert, 1989). Immediately adjacent to roads, it would be expected that vegetation is exposed to wind from passing vehicles. Accelerated wind speeds might increase effective pollutant dose through a reduction in boundary layer resistance at the leaf surface.

Although rainfall is greater (by 5 – 10%) in cities compared with rural areas, this comes mainly in the form of heavy downpours, with rapid run-off into drainage systems. Water availability to vegetation is actually reduced compared with surrounding areas (Gilbert, 1989).

Table 1.1 Average change of climatic parameters in urban compared with surrounding areas (adapted from from Gilbert, 1989; originally from Horbert, 1978).

Climatic parameters	Characteristics	Compared with surrounding area
Air temperature	Annual mean average	0.5 – 1.5° C higher
	On clear days	2 – 6° C higher
Wind speed	Annual mean average	10 – 20% less
	On calm days	5 – 20% more
Relative humidity	Winter	2% less
	Summer	8 – 10% less
Clouds	Overcast	5 – 10% more
Precipitation	Total rainfall	5 – 10% more

1.7.2 Soils

Humans impose rapid transformation cycles on urban land. Regular disturbance means that many urban soils are immature. They often originate from materials derived from previous uses, so that there is great spatial heterogeneity in soil types (De Kimpe and Morel, 2000). Urban soils are generally of a poor structure. Compaction, caused by people and vehicles, reduces their aeration and water permeability and discourages root penetration (Gilbert, 1989).

Soil pH can be shifted in either direction compared with surrounding areas. Alkaline soils can be the result of disturbance bringing unleached material to the surface, or to the release of calcium from building-rubble. Deposition of acidic pollutants can shift pH in the opposite direction (Gilbert, 1989). Pollutants deposited onto urban soils include heavy metals (although these are becoming less important due to the use of catalytic converters), and compounds such as NO_3^- that can act as plant nutrients (e.g. Lovett *et al.*, 2000; Gregg *et al.*, 2003). Nutritional imbalances may result, with an excess of some elements and deficiencies in others. Urban soils have altered microbial comminutes compared with surrounding areas. In the case of symbiotic micorrhizae, this may be due to

enhanced nitrogen input derived from vehicle emissions (Egerton-Warburton and Allen, 2000).

1.8 Interactions of urban pollution with other stresses

1.8.1 Insect herbivory

Exposure of plants to certain air pollutants can predispose plants to attacks by both sucking and chewing insects. Fumigations of plants with NO_2 have often been found to increase insect pest performance (reviewed in Bell *et al.*, 1993). In filtration studies, populations of aphids on plants grown in ambient urban or roadside air can reach much higher numbers compared with controls (Dohmen *et al.*, 1984; Whittaker, 1994). In a transect away from central London, Houlden *et al.* (1990) found large changes in the growth rates of aphids on barley, with reductions in growth rates as NO_2 concentrations declined away from the city.

The effects of urban pollution on insect pests is thought to be mediated through NO_x-induced changes in the host plant's nitrogen metabolism (Viskari et al., 2000c) which improve the plant's nutritional quality. In particular, changes in the amino acid composition of plants exposed to vehicle emissions can be favorable to the growth and development of insect pests (e.g. Bolsinger and Fluckiger, 1989).

1.8.2 Fungal pathogens

From field observations and fumigation studies, SO_2 and O_3 pollution have been shown to alter the incidence of plant fungal pathogens. The intensity of infection by biotrophic pathogens is usually decreased, while for non-biotrophs, both increases and reductions in infection rates have been observed (reviewed in Bell *et al.*, 1993). There is little direct evidence of effects of current urban pollution climates on fungal pathogens, though a recent transect study has pointed to a suppression of infection by tarspot disease of sycamore in urban areas (Jarraud, 2000)

1.8.3 Drought

Plants growing in urban situations often encounter conditions of low water supply, coupled with enhanced nitrogen deposition. In response to increased nitrogen inputs, plants often undergo a reduction in root:shoot ratio, so that the area of transpiring surfaces is increased while that of water-absorbing surfaces is decreased. This would be expected to decrease the plant's tolerance to water stress (NEGTAP, 2000). Where stomatal conductance is increased in response to pollution, transpiration would also be increased, accelerating wilting in conditions of low water supply. In some cases, water stressed plants have been found to be more tolerant to ozone, possibly because drought-induced reductions in stomatal conductance led to reduced uptake of the pollutant and a lower effective dose (e.g. Mills, 2002).

1.8.4 Frost

There is evidence that exposure to pollutants may reduce frost resistance in woody plants (Mansfield and Lucas, 1996). For example, Caporn *et al.* (2000) found enhanced cellular damage (assessed by electrolyte leakage) under acute frost in heather plants exposed to NO_2 and SO_2. However, due to the heat island effect, plants in urban environments do not often encounter frost.

1.9 Critical levels for NO_x

Using information on effects of pollutants on vegetation, attempts have been made to establish air quality control standards for the protection of ecosystems. Critical loads and levels are developed with the aim of protecting the most sensitive components of ecosystems. The UN/ECE and WHO air quality guidelines for total NO_x for all types of vegetation is 30 ppb as an annual mean and this has been adopted as the UK objective (NEGTAP, 2000). This value is exceeded around most urban areas and motorways. The EU Air Quality Daughter Directive states that the air quality objectives need not be applied less than 20 km from large urban areas, or less than 5 km from motorways or areas with a

population above 5000 people. However, sites around cities make up a large land area, including many areas of high conservation value (NEGTAP, 2000).

1.10 The uses of vegetation in cities

Vegetation has the potential to improve urban air quality by intercepting and removing gaseous and particulate pollutants. Trees efficiently trap particles through localized increases in wind speed caused by surface roughness, resulting in reduced boundary layer resistance at the leaf surface and enhanced turbulent deposition (Becket *et al.*, 1998). Gaseous pollutants and particles below 1 μm diameter diffuse across concentration gradients due to Brownian motion (Chamberlain and Little, 1981), and are therefore attracted onto the surface of the substomatal cavity (Thompson *et al.*, 1984).

Tree species differ in their effectiveness as pollutant filters. As mentioned above, surface area and surface roughness, even down to the micromorphological level, are factors, as well as leaf wetness (e.g. Grantz *et al.*, 1997; Farmer, 2002). Deciduous trees are thought to be more pollution-tolerant, since they renew their leaves each year, so decreasing their accumulated load of contaminants (Becket *et al.*, 1998). There is however a possibility of toxic pollutants being returned to the soil after leaf fall, which could cause damage to roots (Kahle, 1993). The most effective pollution-filtering trees are likely to be species that exhibit high rates of stomatal conductance (Good, 1990; Freer-Smith and Broadmeadow, 1996; Becket *et al.*, 1998), whose associated high rates of transpiration would also work against the heat-island effect. To maximise their effectiveness, trees should be positioned close to sources of pollution (Madders and Lawrence, 1981). Several studies have attempted to quantify the air-improving properties of vegetation. McPherson *et al.* (1994) found that trees in Chicago improved average hourly air quality by 0.4%, and in more densely wooded areas by as much as 2.1%. In a mathematical model, Freer-Smith and Broadmeadow (1996) found urban trees to have the potential to take up significant amounts (c. 20% of

exposure concentrations) of O_3 and SO_2 during pollution episodes (reviewed in Becket *et al.*, 1998).

As well as its potential usefulness, urban vegetation also contributes to emissions of both particulates and VOCs. Tree pollen is a form of particulate pollution that can have health effects on people in the form of hey fever (Beckett *et al.*, 1998). Biogenic VOCs can contribute to secondary particle formation, and like anthropogenic VOCs, are potential precursors to O_3 formation (e.g Simpson *et al.*, 1999). This latter role can be significant, particularly in urban areas, where NO_x concentrations are high (Mendoza-Dominguez *et al.*, 2000). However, this effect has been found to be much more important in warm climates such as parts of North America compared with North European urban areas (Owen *et al.*, 2003).

1.11 Aims of the project

A unique opportunity was available with the urban pollution Solardome fumigation system to expose plants to urban pollutants under close-to-natural conditions, but without the constraints of field studies where environmental and pollution effects are difficult to separate.

Specific aims were:

- To create an artificial pollution climate mimicking a mid- to heavily- polluted urban site (roadside/kerbside conditions);
- To gauge long-term consequences of exposure to urban pollution;
- To expose a wide range of ornamental tree and shrub species to the pollution mixtures;
- To investigate broad effects on growth and physiology;
- To examine any interaction between pollution and other stresses commonly encountered in urban situations.

Chapter 2: Materials and Methods

2.1 Exposure system

2.1.1 Solardome glasshouses

The exposure system was situated at the Centre for Ecology and Hydrology's Climate Change Solardome Facility, Abergwyngregyn, North Wales. It comprised four 3.1 m diameter hemispherical glasshouses (Solardome™, Southampton, UK), constructed on an east-west line. This closed chamber system design has previously been used in controlled exposures with SO_2, NO_2 and O_3 (Rafarel and Ashenden, 1991) and CO_2 (Rafarel *et al.*, 1995). Each Solardome was ventilated with air from fan filter units (Roof Unit Group, West Midlands, UK) with charcoal/Purafil™-filtered air at 0.58 complete air changes per minute. The air was delivered via a perforated ring of polythene tubing (Bailey Polythene, Ventnor, Isle of Wight) running the inner circumference of each Solardome at ground level. The perforations, in three parallel rows, were 12 mm in diameter. Air exits the Solardomes via a ventilation cowl at the top. Urban pollution mixtures were simulated by introducing exhaust emissions from a diesel generator at a target concentration of 100 ppb total NO_x. The fumigation was constant, 24 hours per day, 7 days per week throughout the growing season.

2.1.2 Pollution production and delivery

Exhaust emissions from a 4 kw diesel generator (Generac™ ED 400, Lombardini, Italy) were fed into the air streams entering two Solardomes. Photographs of the generator and delivery system and of the Solardome glasshouses are shown in Figure 2.1.

A feasibility study for this system (Moonen *et al.*, 1999), which also tested a petrol generator and a car engine, had shown that exhaust from a diesel generator produced a gas mixture closest to that of a typical polluted urban atmosphere. Rebated Texaco Gas Oil was used as fuel for the generator.

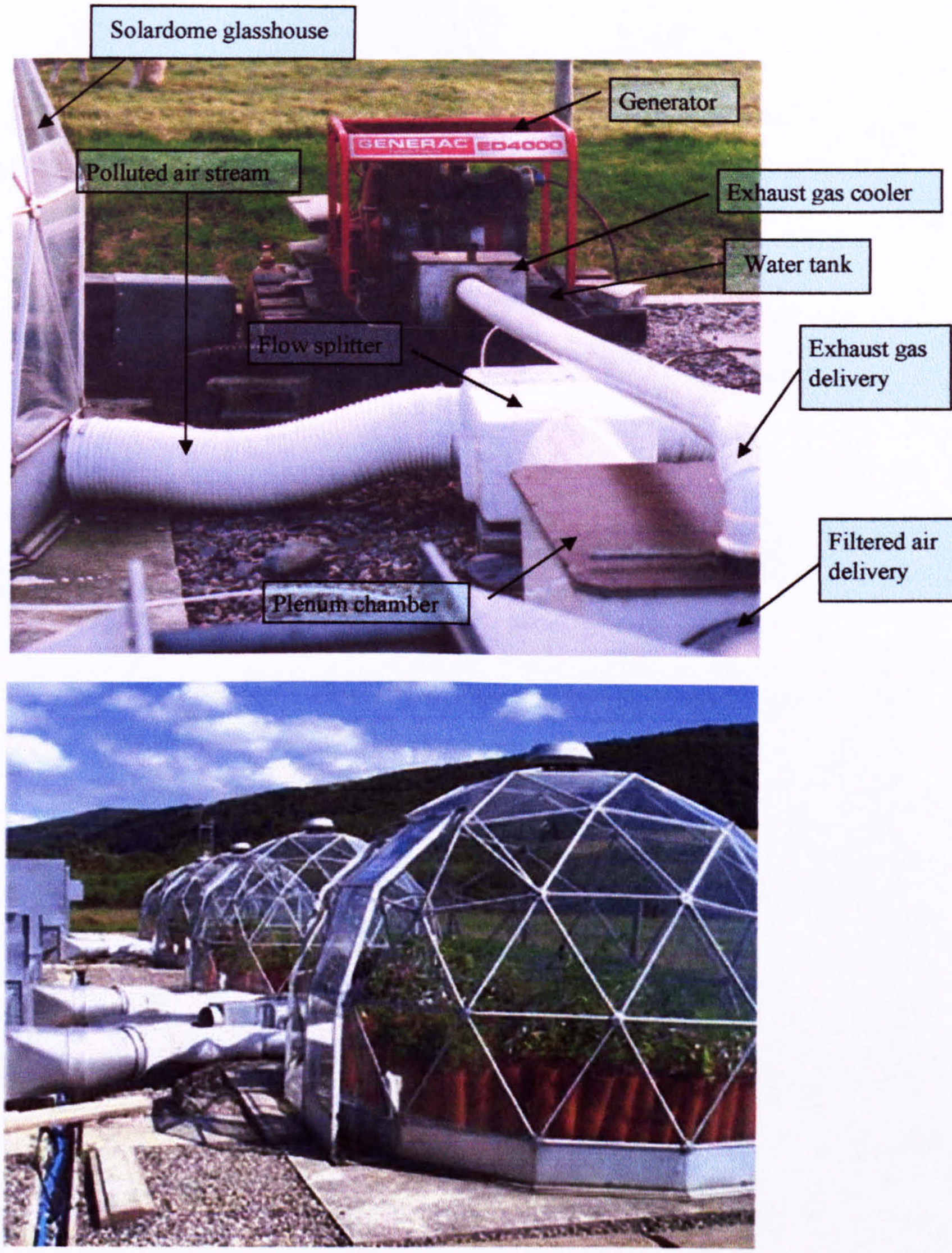


Figure 2.1 Photographs of the pollution delivery system and plants being fumigated in the Solardome glasshouses.

Exhaust output from the generator was split into two streams. The first supplied the treatment Solardomes with exhaust gases, and the second carried away excess emissions. The pipe carrying away the excess gasses was fitted with an adjustable back-pressure valve, allowing control over the amount of polluted air entering the Solardomes.

The stream destined for the Solardomes was first piped to a stainless steel vessel (0.33 m x 0.30 m x 0.31 m) which was partially submerged in a cooling tank. This caused the hot exhaust gases to cool and condense and the heavier oil particles to drop out into the waste exhaust stream. The gas mixture then traveled along 0.08 m diameter piping to a plenum chamber, constructed from a PVC water tank (0.49 m x 0.55 m x 0.74 m) where it was mixed with the filtered air stream. Entry of the exhaust gas mixture into the plenum chamber was via four holes in the delivery pipe, each of 5 mm diameter, close to and perpendicular to the input for the filtered air. Further mixing of the exhaust gasses with the filtered air within the plenum chamber was encouraged by baffles. From the plenum chamber, the gas mixture was delivered to a flow splitter, constructed from two small PVC tanks (0.28 m x 0.43 m x 0.60 m), where it was divided into two equal flows and fed into the Solardomes via 0.18 m diameter corrugated piping.

The remaining two Solardomes were clean air controls. For these chambers, filtered air was delivered directly to a similar flow splitter to that described above via 0.24 m diameter corrugated piping, and was then fed into the Solardomes.

2.1.3 Control of pollutant concentrations

Concentrations of pollutants entering the treatment Solardomes were regulated by a control valve, which adjusted the amount of exhaust being dumped in the waste stream. NO_x levels were continually monitored by a 200A chemiluminescent NO_x analyser (Advanced Pollution Instrumentation, USA), and used as an indicator of exhaust gas concentrations. The target NO_x

concentration was 100 ppb. In 2000, the control valve was adjusted by hand when NO_x concentrations drifted from 100 ppb. In 2001, a specifically designed motorised guillotine valve (designed by P. Hadfield of CEH, Bangor) was installed. An analogue signal from the NO_x analyser was relayed to a computer programme written in LabVIEW (National Instruments Corporation, Texas, USA), via FieldPoint modules (National Instruments Corporation, Texas, USA). The programme converted the signal into a ppb value for total NO_x. When this value fell below 90 ppb or rose above 110 ppb, a signal was sent via the FieldPoint modules to adjust the valve accordingly. This negative feedback system was effective in maintaining the NO_x concentrations close to the target of 100 ppb.

2.1.4 Comments on changes to the exposure system

In 2000, the system was still rudimentary. Its fuel storage tank did not have the capacity to keep the generator running for a full day, so that there were often periods in the early morning when the generator stopped running until the fuel could be replaced. The pollution concentrations were controlled manually, and often drifted away from the target of 100 ppb NO_x. As the system became more sophisticated, the control of pollutant concentrations was greatly improved for the 2001 experimental season. Between the 2001 and 2002 experimental seasons, a new generator was fitted, with the effect of altering the NO:NO_2 ratio in the exhaust gas mixture, and increasing the concentrations of benzene and toluene.

2.1.5 Environmental conditions

The conditions of temperature, humidity and light inside the Solardomes have been well characterised (Rafarel and Ashenden, 1991). The Solardome chambers have been shown to reduce irradiance in the PAR by 13-25% compared with ambient, depending on weather conditions. Temperature is elevated by between 2.4 °C on an overcast day to 5 °C on clear, sunny days when the external temperature is above 24 °C (Rafarel and Ashenden, 1991).

2.1.6 Methods of pollution monitoring in the Solardomes

2.1.6.1 Nitrogen oxides

NO_x were continually analysed (every four minutes) in one of the treatment Solardomes using a model 200A chemiluminescent NO_x analyser (Advanced Pollution Instrumentation, USA). Data were logged on a Datahogg datalogger (Skye Instruments Ltd., Powys, UK). Calibration of the instrument is carried out against traceable mixtures of NO and NO_2.

2.1.6.2 VOCs

Combustion processes and subsequent cooling of exhaust gases produce a variety of VOC species. Benzene and toluene were used as indicator species, and were monitored for several days at a time in August, October and November 2001 and between February and August 2002. Measurements were made using standard thermal desorption tubes (4 inch x 1/4 inch stainless steel, Supelco, USA). The tubes were packed with Chromosorb-106, a polymer adsorbant of low to middle boiling point volatiles up to 250°C. Air was drawn through the tubes using a low-volume pump (100ml min^{-1}) for several hours and the volume of air sampled determined by gas meter. Exposed tubes were sealed and sent to J. N. Cape and J. Binnie at CEH, Edinburgh, Bush Estate, Penicuik, Midlothian, where they were thermally desorbed using a Perkin-Elmer ATD-400 Cold Trap (Perkin Elmer, USA). Analysis was by gas chromatography and mass spectroscopy (Hewlett Packard, Canada. Models 5890A series 2 and 5972, respectively) with specific ion detection (m/z 78 and 91 for benzene and toluene, respectively). Calibration was carried out using Supelco VOC mix in methanol, further diluted in methanol. Blank tubes were sent to/from the Solardome facility and anlaysed to test for contamination during transit.

2.1.6.3 HONO

HONO measurements were taken continuously over two week periods at intervals between October 2001 and June 2002. Air was sampled using a low volume pump (flow rate 0.35 l min^{-1}) onto two tubular denuders in series, coated

with sodium carbonate. The technique relies on the diffusion of HONO on the walls of the denuders. Total air volume pasing through the denuders was measured using a gas meter. Denuders were returned to J. N. Cape and J. Binnie at CEH, Edinburgh, Bush Estate, Penicuik, Midlothian for analysis. HONO trapped on the walls of the denuder reacts to form nitrite, which is extracted and analysed by ion chromatography (e.g. Febo *et al.*, 1996). The first denuder gives the concentration of nitrite produced from HONO plus a small amount of nitrite from NO_2. The second denuder gives nitrite produced from NO_2 only. By subtracting the value for NO_2 from the value for HONO + NO_2, the amount of HONO can be calculated. For chromatographic analyses, control samples consisting of known solutions of anions were analysed every seven samples in order to re-calibrate the ion chromatograph. Blank tubes were sent to/from the Solardome facility and anlaysed to test for contamination during transit.

2.1.6.4 Particulates

Particulate concentrations were measured by K. Bignal of Bradford University using a 3-stage particle impactor (Dekati Ltd, Finland). The impactor can classify particles present into three size classes: 0.7 μm - 2.5 μm, 2.5 μm – 10 μm and >10 μm. The impactor accurately classifies the three size classes to ± 2.8% as long as the nominal flow rate, 10 l min^{-1}, is known to ±5.0%. The impaction substrates were coated with exactly 4.0 μl of a saturated solution of Apiezon-L vacuum grease (Apiezon Products, UK) dissolved in toluene at 25° C. The coated substrates were stored in a dessicator for a minimum of 24 h prior to exposure, along with the final stage filters (Whatman® glass microfibre filters GF/F, UK). Particulate concentrations were determined gravimetrically by weighing the substrates and filters before and after exposure. The exposed substrates and filters were then returned to the dessicator for a further 24hr period and weighed again. All weights were taken using a 3 g - 0.000 mg microbalance (Sartorious MP5, Germany). The weigh pan of the balance was modified to allow accurate determination of the large final filters (47mm) used in

the impactor. The flow rate for the pump was set and checked using a DryCal™ primary airflow meter (BIOS, USA).

2.1.7 Results of pollution monitoring in the Solardomes

Concentrations of pollutants monitored in the Solardomes are presented in Table 2.1, and compared with available 2002 data from typical urban background, roadside and kerbside sites. Definitions of this classification of sites are given in Table 2.2.

2.1.7.1 Nitrogen oxides

In the 2000 experimental season, total NO_x concentrations in the exhaust gas mixture showed great variation, and were generally lower than the target of 100 ppb. Over three months of monitoring shown in Figure 2.2, the average NO_x concentrations were 90.65 ppb, with an average NO:NO_2 ratio of 1.67.

The data for 2001 (Figure 2.3) show how effective the system had become at maintaining the NO_x levels close to the target. The average over the experimental season was 96.05 ppb, with an NO:NO_2 ratio of 1.44. During the 2002 experimental season, the NO_x concentration was 92.2 ppb (Figure 2.4). The NO:NO_2 ratio changed considerably compared with 2001, increasing to 1.94.

In these fumigations, the total NO_x concentration used was intended to represent that of a mid- to heavily-polluted urban site, where maximal hourly averages often exceed 100 ppb. Comparison of NO_2 concentrations in the Solardomes with those of roadside and kerbside urban sites shows a close fit with kerbside concentrations for this pollutant (Table 2.1). Between the three years of

Table 2.1 Pollutant concentrations in polluted Solardomes compared with typical 2002 concentrations from urban sites. The annual average urban concentrations are taken from the UK National Air Quality Information Archive (www.airquality.co.uk). The database contains data and statistics from the monitoring networks operated on behalf of the DEFRA (Data from: [1]London, [2]Birmingham, [3]Leeds, [4]Cardiff, [5]Glasgow, [6]Northampton, [7]Stockton-on-Tees; [8]Wellburn, 1990).

Year of study/ type of site	NO (ppb)	NO2 (ppb)	NOx (ppb)	NO:NO2 ratio	Toluene (ng l^{-1})	Benzene (ng l^{-1})	HONO (ppb)	PM 10 (µg m^{-3})	PM 2.5 (µg m^{-3})
2000	57.42 ±4.94	35.51 ±3.62	90.65 ±8.37	1.67 ±0.07	-	-	-	-	-
2001	56.73 ±1.63	39.36 ±1.22	96.05 ±2.75	1.44 ±0.001	0.86 ±0.07	4.59 ±0.32	2.72 ±0.28	38.67 ±3.93	36.57 ±4.01
2002	60.40 ±2.37	31.71 ±1.28	92.20 ±3.06	1.94 ±0.08	5.11 ±0.23	7.28 ±0.31	5.43 ±0.18	-	-
Background		[2]12.36 ±1.04		[8]1.00	[4]1.83 ±0.03	[3]0.69 ±0.02		[1]19.34 ±0.01	[1]9.56 ±0.06
Roadside		[1]22.50 ±1.45		[8]4.00	[5]4.67 ±0.05	[2]2.07 ±0.11		[6]21.87 ±0.55	[1]13.66 ±0.08
Kerbside		[1]30.60 ±2.40			[1]9.42 ±0.09	[7]2.15 ±0.12		[1]34.21 ±0.19	[1]21.48 ±0.11

Table 2.2 Description of the different types of urban sites monitored for pollution concentrations by DEFRA (adapted from www.airquality.co.uk)

Site type	Characteristics
Urban Background	Urban locations distanced from sources and broadly representative of city-wide background concentrations e.g. elevated locations, parks and urban residential areas.
Roadside	Sites with sample inlets between 1m of the kerbside of a busy road and the back of the pavement. Typically this will be within 5m of the kerbside. Sampling heights are within 2-3m.
Kerbside	Sites with sample inlets within 1m of the edge of a busy road. Sampling heights are within 2-3m.

experiments, average concentrations in the Solardomes ranged from 31.71 ppb to 39.36 ppb, compared with 30.60 ppb for a typical kerbside site. The exposure did not, however, mimic the diurnal variation in pollutant concentrations that exists in real urban situations. Additionally, NO:NO_2 ratios in the Solardome atmosphere (1.67 in 2000, 1.44 in 2001 and 1.94 in 2002) were quite different to those typically found in roadside air (NO:NO_2 of around 4.0; e.g. Wellburn, 1990), and were in fact closer to typical urban background ratios (around 1.0; e.g. Wellburn, 1990).

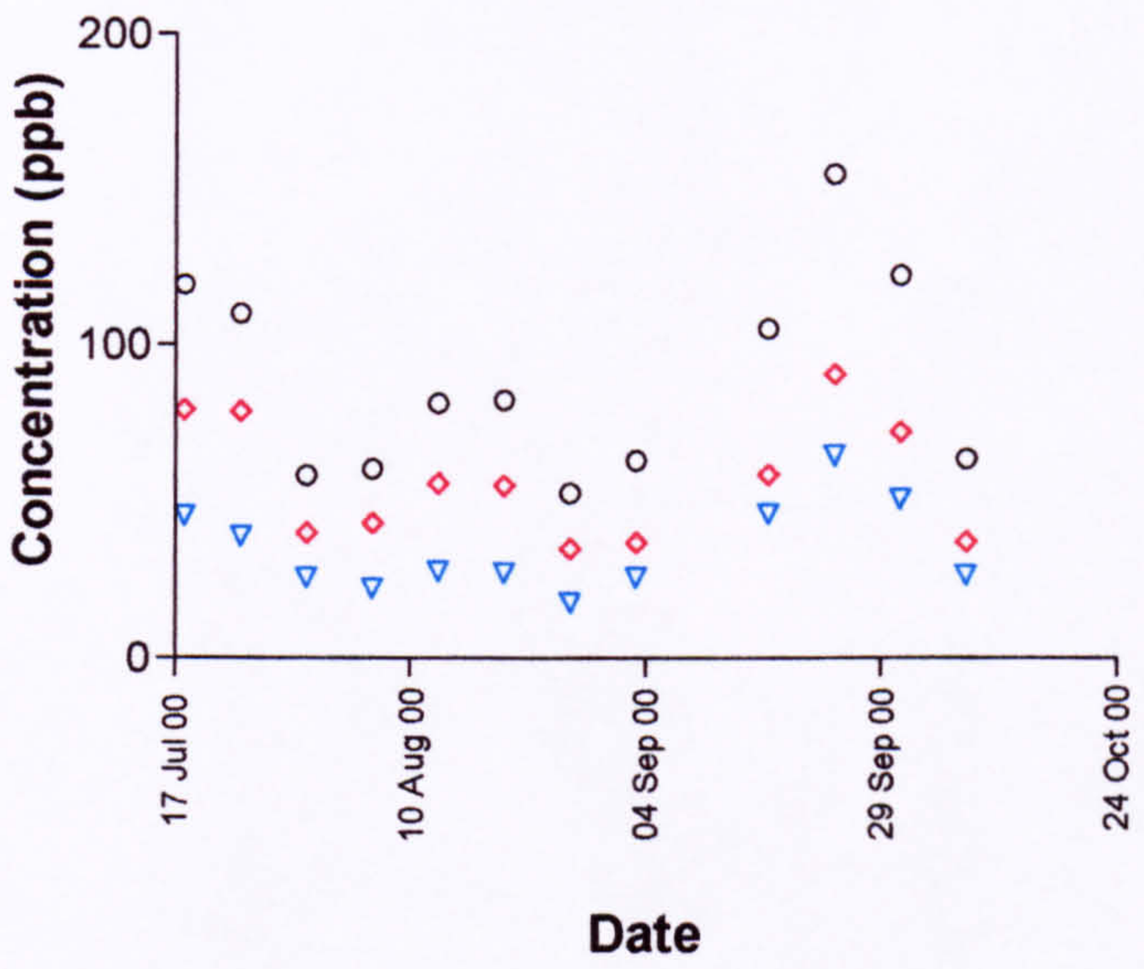


Figure 2.2 Weekly average concentrations of NO (◊), NO_2 (▽) and total NO_x (○) in a polluted Solardome between July and October 2000. Gaps are where the generator was not running.

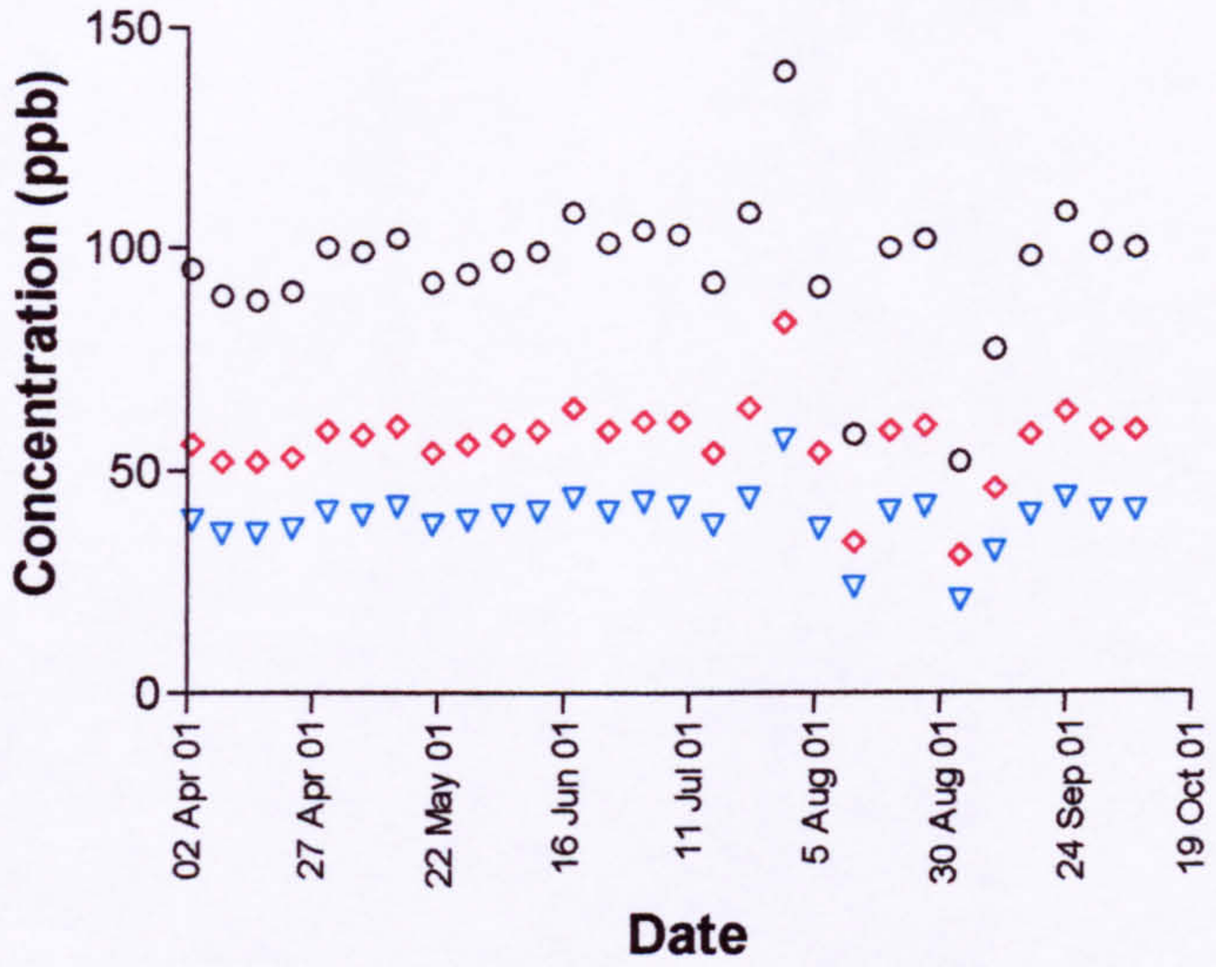


Figure 2.3 Weekly average concentrations of NO (◊), NO_2 (▽) and total NO_x (○) in a polluted Solardome between April and October 2001.

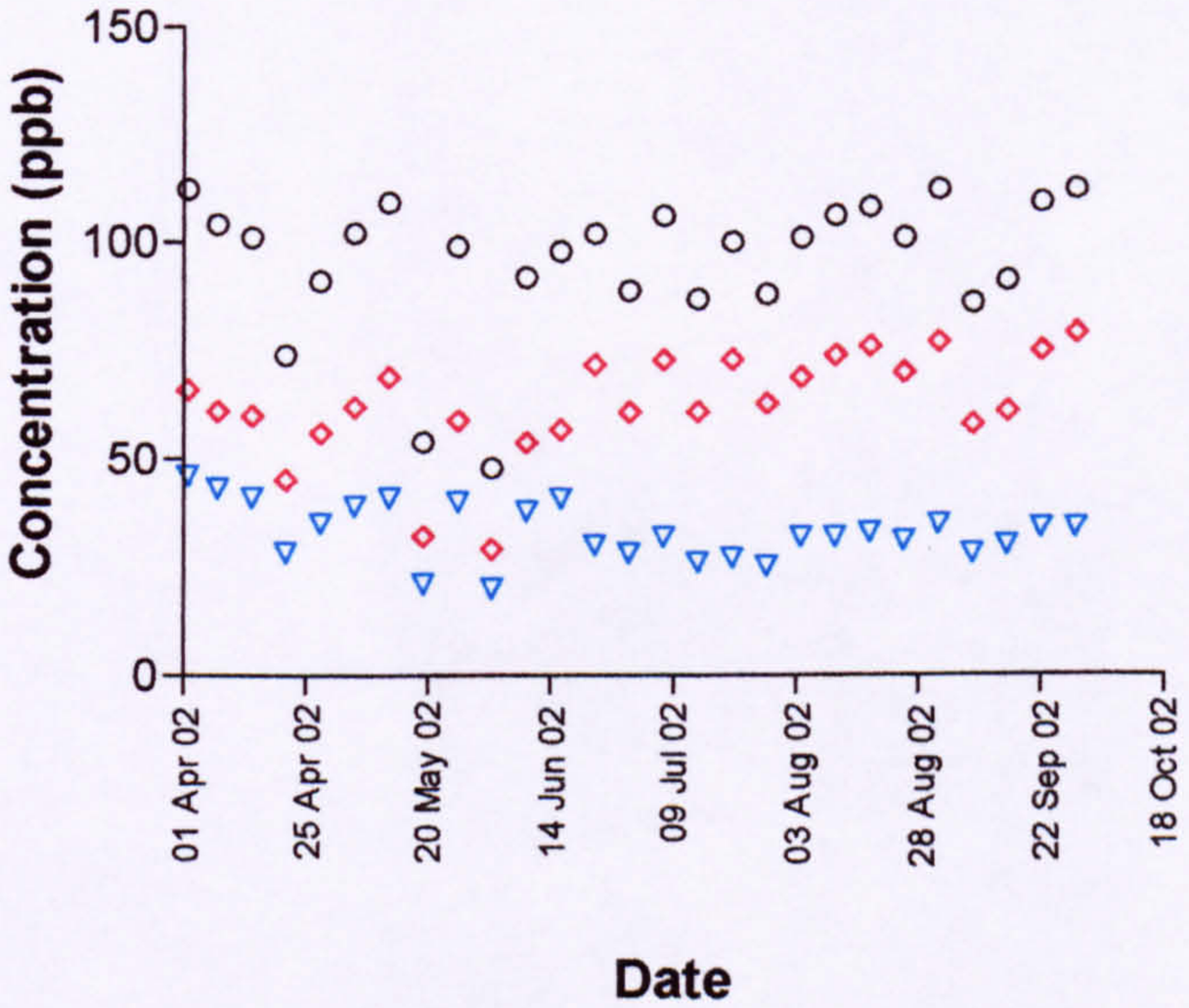


Figure 2.4 Weekly average concentrations of NO (◊), NO_2 (▽) and total NO_x (○) in a polluted Solardome between April and October 2002.

2.1.7.2 VOCs

Benzene concentrations in the Solardome atmosphere (Figures 2.8 and 2.9) were elevated compared with those in real urban situations. In 2001, the average of all the measurements of benzene made in exhaust gas-polluted Solardomes was 4.6 ng l^{-1}, compared with concentrations at kerbside sites of around 2.15 ng l^{-1} (Table 2.1). With the change in the system between the 2001 and 2002 experimental seasons, the benzene concentrations in the Solardomes increased, having an average of 7.28 ng l^{-1}.

In 2001, toluene concentrations in the Solardomes (Figure 2.10) were low compared with those found in urban roadside atmospheres (an average of 0.86 ng l^{-1} in the Solardomes compared with roadside concentrations of 4.67 ng l^{-1}). In 2002, the average concentrations in the Solardomes were close to those at the roadside, at 5.11 ng l^{-1} (Figure 2.11; Table 2.1).

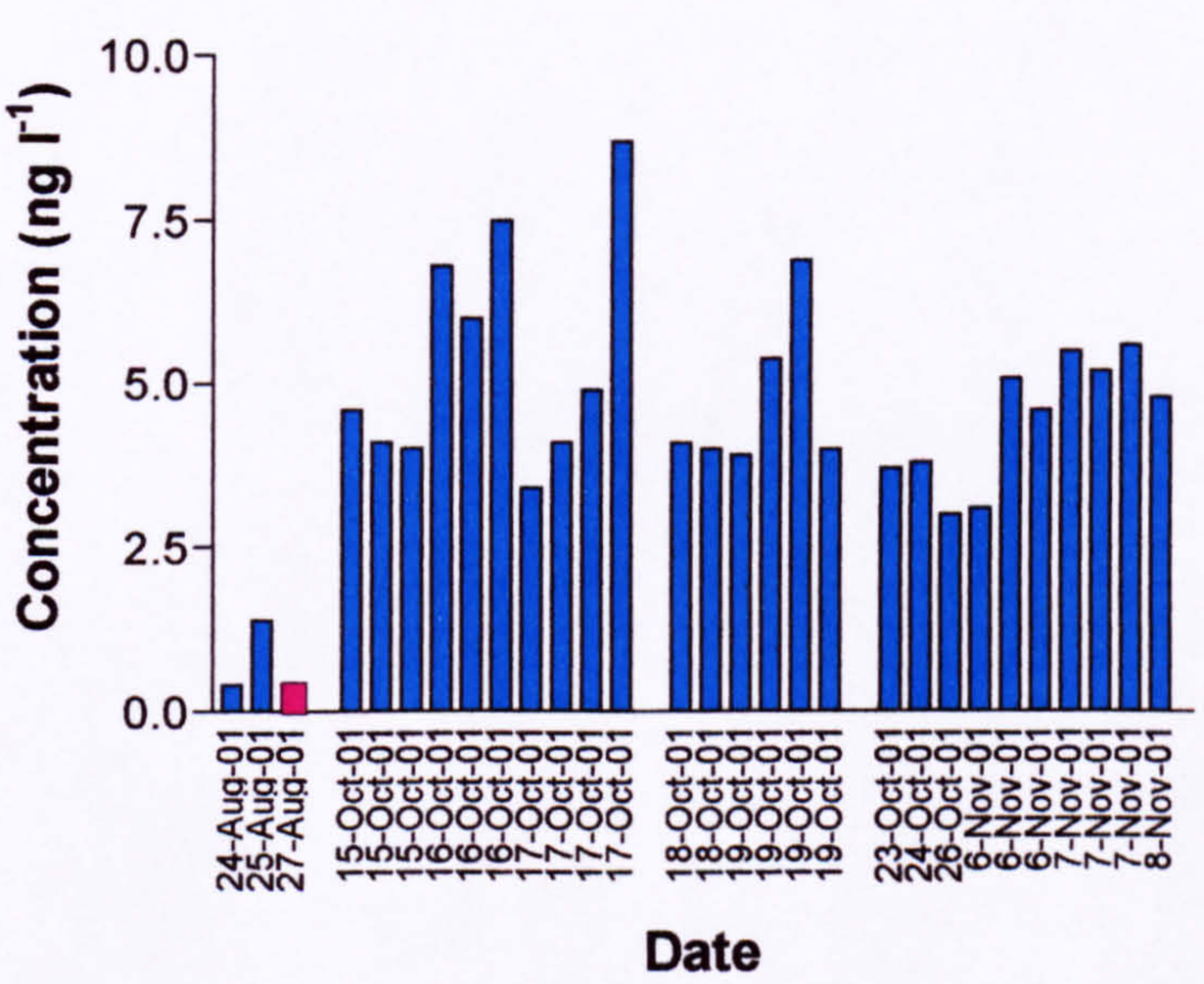


Figure 2.8 Concentrations of benzene in exhaust gas-polluted Solardomes (■) and in CFA (■) in 2001. Values represent the mean of two measurements.

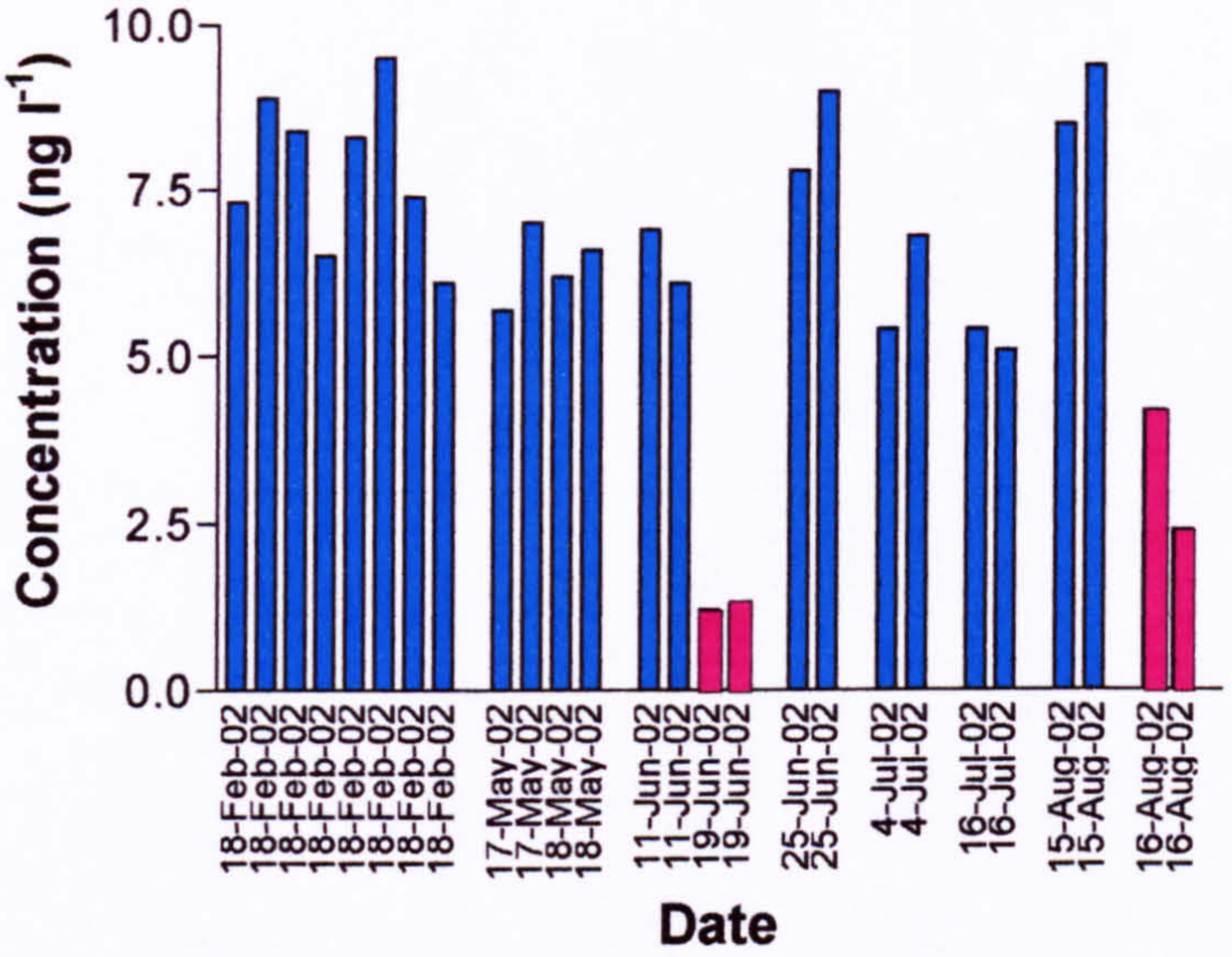


Figure 2.9 Concentrations of benzene in exhaust gas-polluted Solardomes (■) and in CFA (■) in 2002. Values represent the mean of two measurements.

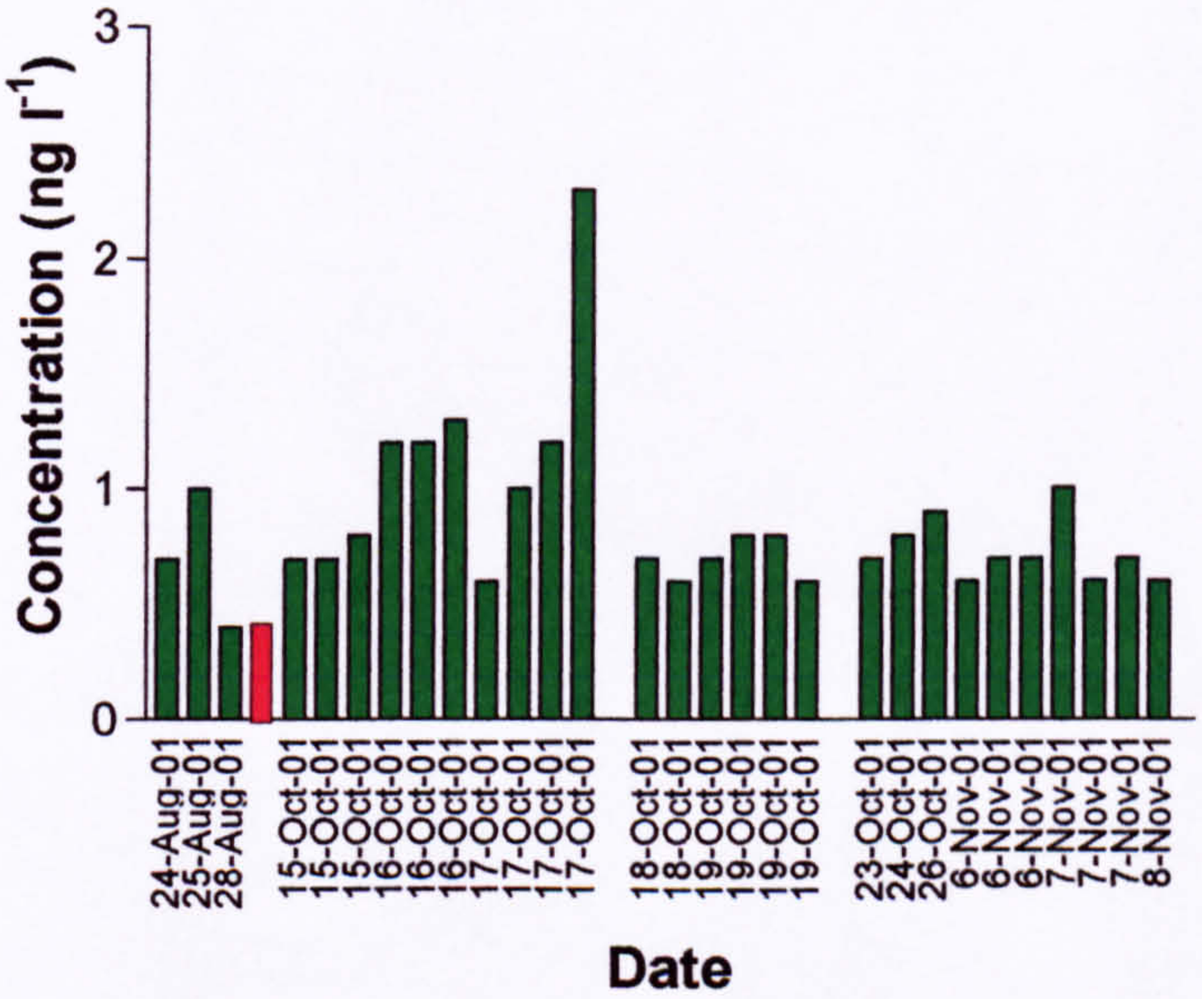


Figure 2.10 Concentrations of toluene in exhaust gas-polluted Solardomes (■) and in CFA (■) in 2001. Values represent the mean of two measurements.

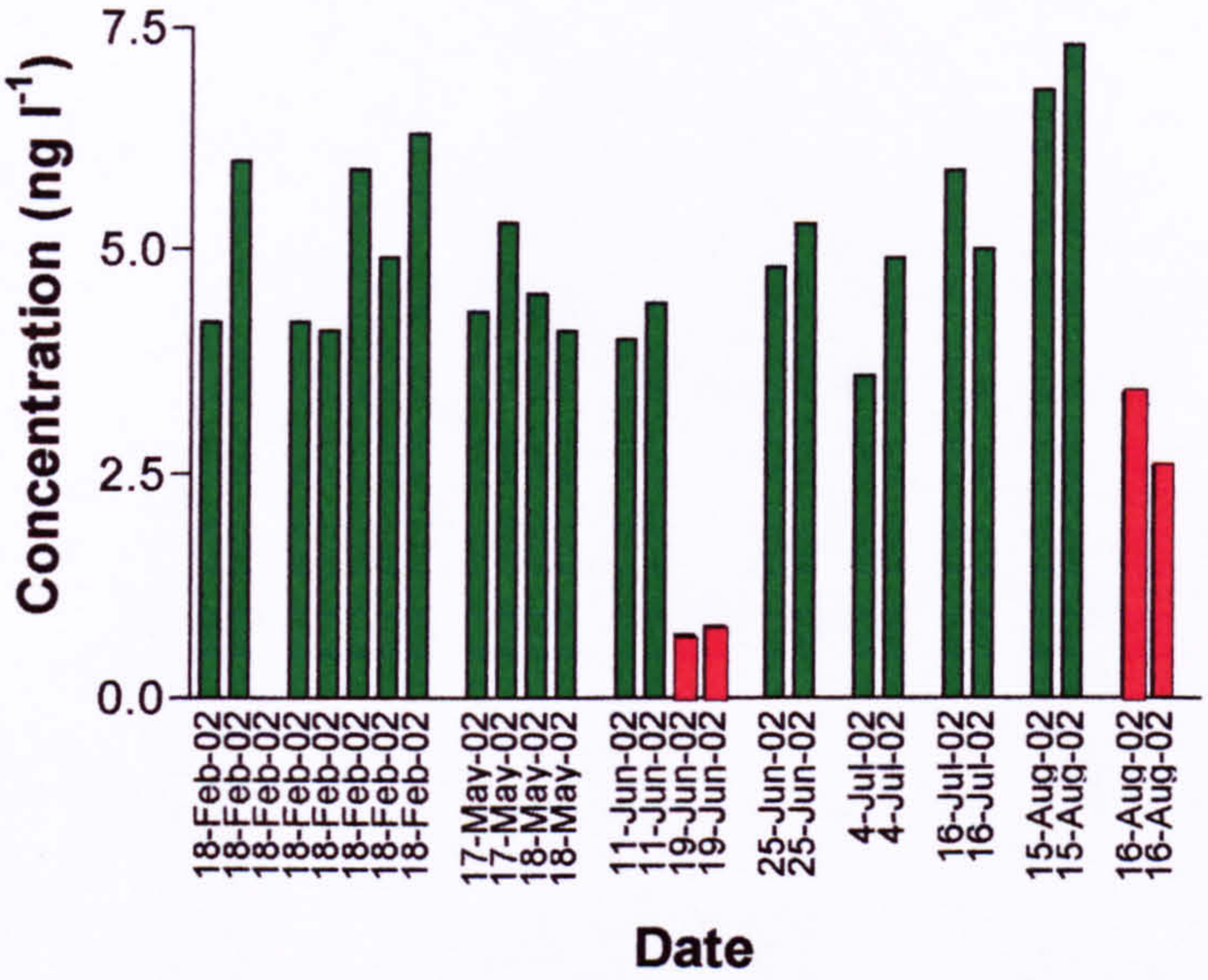


Figure 2.11 Concentrations of toluene in exhaust gas-polluted Solardomes (■) and in CFA (■) in 2002. Values represent the mean of two measurements.

2.1.7.3 HONO

Concentrations of HONO in the Solardomes between October 2001 and June 2002 are given in Figure 2.12. The average for all measurements made in 2001 is 2.72 ppb, and in 2002 5.43 ppb (Table 2.1). Few data exist on HONO concentrations in cities, but some studies have pointed to typical nighttime concentrations of around 3 ppb (e.g. Reisinger, 2000). Nighttime peaks of up to 10 ppb have been recorded (e.g. in Birmingham, UK; Notholt *et al.*, 1992). These figures are for the time of HONO's diurnal cycle when concentrations reach their maxima. In comparison with typical values in urban atmospheres, the concentrations in the Solardomes were high. This could be due to the lack of O_3 formation in the Solardomes, a process that normally destroys HONO molecules.

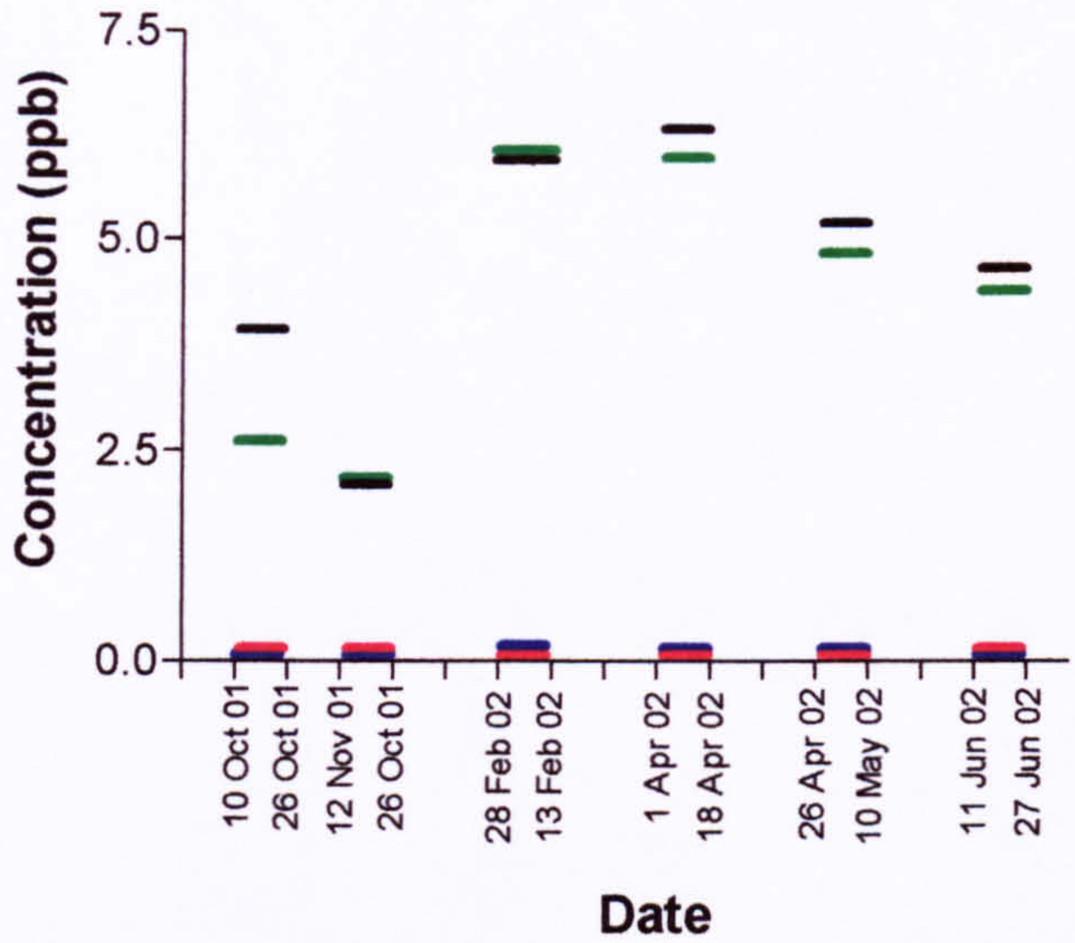


Figure 2.12 Concentrations of nitrous acid (HONO) in exhaust gas-polluted Solardomes 1 and 2 (— and —, respectively), and in CFA Solardomes 1 and 2 (— and —, respectively). Values represent the mean of two measurements.

2.1.7.4 Particulates

Table 2.3 shows the concentrations of different size classes of particulates in the Solardomes. The average concentrations of particles between 2.5 μm and 10 μm diameter was 1.75 μg m^{-3}. Total PM_{10} concentrations were 38.67 μg m^{-3}. Maximal hourly averages of 200-320 μg m^{-3} PM_{10} occur in most large cities (DoE, 1995), with annual averages of around 20 μg m^{-3} for roadside sites and around 35 μg m^{-3} for kerbside sites (Table 2.1). The concentrations in the Solardomes were therefore comparable to kerbside levels. Concentrations of $PM_{2.5}$ were elevated in the Solardomes compared with actual urban atmospheres. The Solardomes had an average $PM_{2.5}$ concentration of 36.57 μg m^{-3} compared with around 20 μg m^{-3} at a typical highly-polluted site (Table 2.1). This could be due to the source of the emissions being exclusively diesel exhaust.

Table 2.3 Particulate concentrations in exhaust gas-polluted Solardomes in 2001.

Date	PM >10 μm diameter ($\mu g\ m^{-3}$)	PM 2.5 - 10 μm diameter ($\mu g\ m^{-3}$)	PM 0.7 - 2.5 μm diameter ($\mu g\ m^{-3}$)
14th June 2001	1.79	2.71	24.33
12th Oct 2001	1.39	2.39	48.08
15th Oct 2001	3.09	2.12	31.83
17th Oct 2001	1.19	1.40	40.48
23rd Oct 2001	1.29	1.88	38.16

2.2 Plant Measurements

2.2.1 Growing conditions

Plants were potted in Gem™ John Innes "Number 2" compost in 10 cm diameter, 100 cm deep pots. Apart from experiments where water or nutrients were controlled, plants were watered/fertilized as required.

2.2.2 Stomatal conductance

Stomatal conductance to water vapour was measured for the second-eldest leaf of each plant using an AP4 porometer (Delta-T Devices, Burwell, Cambridge, UK).

2.2.3 Gas exchange and Photosynthesis

Gas exchange and photosynthesis measurements were made on the second-eldest leaf using an infra-red gas analyser (Ciras II, PP Systems, Hitchin, UK), linked to an automated Parkinson leaf cuvette (model AUTO-PLC-B, PP Systems, Hitchin, UK). Light-saturated rate of CO_2 assimilation (A_{sat}) was measured at $800 \pm 2\ \mu mol\ m^{-2}\ s^{-1}$ light, a leaf temperature of $25 \pm 2°C$ and 360 ppb CO_2.

2.2.4 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were made on the second eldest leaf using a Handy Plant Efficiency Analyser (Hansatech Instruments Ltd., Kings Lynn, UK). The maximal quantum efficiency of Photosystem II (Fv/Fm) and "AREA" values (described below) were determined after 40 minutes dark-adaptation followed by exposure to a saturating light pulse (3000 μmol m^{-2} s^{-1} for 2 s).

Chlorophyll fluorescence has been used in many studies as a non-destructive method for monitoring stress or selecting plants with stress resistance (drought, heat, waterlogging, pollutants, freezing (e.g. Bilger, Schreiber and Langer, 1984; Conroy *et al.*, 1986; Ögren, 1990; Fracheboud *et al.*, 1999; Clark *et al.*, 2000 a and b). Light energy can be used in three ways: in photosynthesis, or if the light is too high or the plant is stressed, the excess energy is dissipated as fluorescence (i.e. it is re-emitted at a longer wavelength) or as heat (Krause and Weis, 1991). When a leaf is kept in the dark all residual dissipated energy is used and the fluorescence is low (Fo). A short flash with a bright light increases the fluorescence to a maximum (Fm) because the chlorophyll in the leaf cannot use all of the energy. The difference between Fm and Fo is Fv, the variable fluorescence. Fv/Fm is a measure of the proportion of the maximum fluorescence that is used in photosynthesis and it averages around 0.83 in healthy plants. A ratio less than this occurs under stress. Most authors investigating stress have used Fo, Fv, Fm and Fv/Fm for measurements but some have used complex, derived indices such as Strasser's JIP test (Strasser, Srivasatava and Govindjee, 1995). Clark *et al.* (2000b) found a relationship between ozone exposure, biomass, symptoms and a derived index that they called the Fluorescence Performance Index.

Investigations by Davison *et al.* (unpublished) on fluorescence in *Rudbeckia laciniata* (cutleaf coneflower) growing in the field showed that most of the

Hansatech Plant Efficiency Analyser output parameters were strongly correlated with each other. Also, in coneflower, Fv/Fm was not very sensitive to ozone so the authors used one of the simpler indices that proved to be stress sensitive: the area above the transient curve between Fo and Fm (AREA). It is related to the pool size of PSII electron transport acceptors on the reducing side of photosystem II, and in coneflower it is correlated with the light-saturated rate of net photosynthesis. The Hansatech PEA literature (http://www.hansatech-instruments.com) points out that one reason the AREA measurement is a useful parameter is because it indicates *any* change in the shape of the induction kinetic between Fo and Fm which would not be evident from the other parameters e.g. Fo, Fm, Fv/Fm. Hence it is was chosen for the present work.

2.2.5 Droplet contact angle measurements

Leaves were excised and taken immediately to the laboratory for droplet contact angle measurements. A 5 µl droplet of distilled water was placed onto the adaxial surface of the leaf using a HPLC syringe (SGE, Melbourne, Australia) and viewed using a binocular microscope fitted with a protractor graticule. The contact angle was determined immediately the droplet was placed onto the leaf surface.

The angle of contact of the water droplet with the leaf surface gives a measure of the wettability of the surface. Low angles are indicative of high wettability, and a tendency to form water films as opposed to droplets on the leaf surface (Schreuder *et al.*, 2001). Droplet contact angles indicate changes in the outer layer only of the cuticle, i.e. the epicuticular waxes. The more hydrophobic the surface, the greater the angle of contact made by the droplet (Figure 2.13).

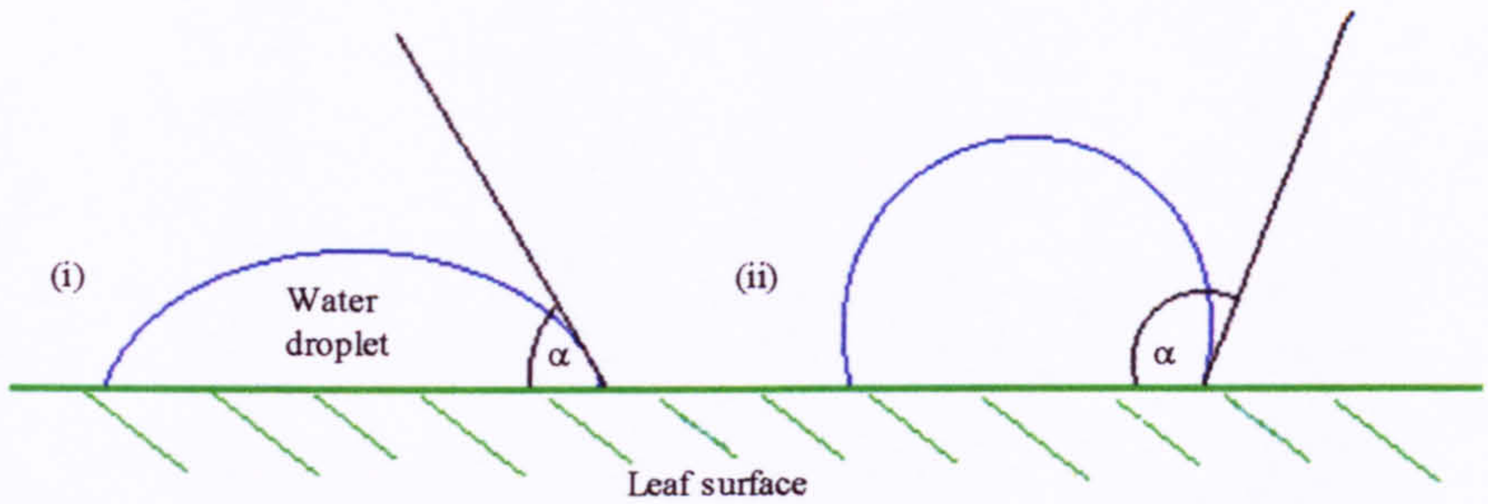


Figure 2.13 Contact angle of a water droplet on (i) a wettable leaf surface (angle <90°) and (ii) a hydrophobic leaf surface (angle >90°). Adapted from Cape, 1983.

2.2.6 Leaf drying rates

The rate of water loss of excised leaves has been used as an indicator of the effects of air pollutants on plants (e.g. Neighbour *et al.*, 1988 looking at the effects of SO_2 and NO_2 on birch; Barnes *et al.*, 1990 looking at the effects of O_3 on Norway spruce). Pollution-induced increases in rates of water loss may indicate impairment of stomatal control and/or increased cuticular diffusion (Cape and Percy, 1996). The rate of water loss can be expressed in terms of the relative water content (RWC) of leaves over time.

Plants were well watered in the evening, and on the following morning a recently expanded leaf was removed from each plant. The fully hydrated leaves were taken immediately to an air-conditioned room (20°C; 50% relative humidity), their petioles sealed with paraffin jelly, and the weights of individual leaves recorded. Each leaf was transferred to a petri dish and re-weighed at intervals over the course of the day, and once again on the following day. Samples were then placed in an oven at 80°C for 48 h, and re-weighed to obtain the dry weight. The rate of water loss from leaves was calculated by relative water content of the leaves (RWC):

$$RWC(t) = \frac{(m(t) - m_d)}{(m_f - m_d)}$$

where $RWC(t)$ is the relative water content of the leaf at time t, $m(t)$ is leaf mass at time t, m_f is fresh mass (fully hydrated leaves) at $t = 0$, and m_d is leaf dry mass. (from Cape and Percy, 1996).

2.2.7 Water status

Water status is measured using water potentials (ψ). This is the sum of pressure potential (ψ_p) and osmotic potential (ψ_o). (The matric potential is also a component, but is considered to be negligible in plants). Pressure potential is caused by the pressure exerted upon the cell by the cell wall, and is almost always a positive value. Osmotic potential is a negative value, and is due to solute molecules in the cell, with a high concentration of solutes causing a greater tendency for water to flow into the cell (i.e. a more negative osmotic potential). The water potential of pure water is arbitrarily set to 0 MPa. In comparison to this standard, leaf water potentials range from <0.1 to about -5 MPa. Water potential becomes more negative as leaves dry, since water loss causes the solutes in the cells to become more concentrated, making the osmotic potential more negative. Upon wilting, there is no longer a pressure potential exerted by the cell wall and $\psi = \psi_o$.

Leaf water potential was measured using Merrill thermocouple leaf-cutter psychrometers with a Model 85 meter (J. R. D. Merrill Speciality Equipment, Logan, Utah, USA). From each leaf, two leaf discs of 4 mm diameter were collected in the vessel of each psychrometer cell. The cells were placed into a water bath at 25°C for 2 hours, after which the water potentials of the samples were measured.

2.2.8 Stable carbon isotope discrimination

The carbon isotope ratio of leaves can give an integrated measure of stomatal conductance over time. The $^{13}C/^{12}C$ ($\delta^{13}C$) isotopic ratio of CO_2 in the atmosphere is altered upon uptake and metabolism into plant tissues due to discrimination against the heavier isotope (Farquhar and Richards, 1984). This arises due to differential diffusion through the stomata (discrimination against ^{13}C = 4.4 ‰), and fractionation by RUBISCO (discrimination c. 27-28 ‰). The stable carbon isotope ratio of a sample is conventionally measured relative to the PDB standard (a calcium carbonate sample from a limestone fossil at Pee Dee, South Carolina). The PDB standard was assigned a $\delta^{13}C$ ($^{13}C/^{12}C$) of 0 ‰. Compared with this value, plants exhibit negative values of $\delta^{13}C$. However, it is more appropriate with plant material to express discrimination by the leaf as isotopic composition relative to the source carbon in the atmosphere. Farquhar and Richards' (1984) carbon isotope discrimination ($\Delta^{13}C$) is a positive value, since plants discriminate positively against ^{13}C. It is calculated as follows (from Fessenden and Ehleringer, 2002):

$$\Delta^{13}C = ((\delta^{13}C_a - \delta^{13}C_p) / (1000 + \delta^{13}C_p)) \times 1000$$

where $\delta^{13}C_a$ is the isotopic ratio of the air and $\delta^{13}C_p$ is the is the isotopic ratio of the plant, relative to the PDB standard.

Plant tissue for carbon isotope analysis was oven dried at 80°C and ground in a mill. Samples (1 mg) were analysed by G. Taylor at the Biomedical Mass Spectrometry Unit at the University of Newcastle. The $\delta^{13}C$ values obtained were converted to $\Delta^{13}C$ values.

2.2.9 Nitrate reductase (NR) activity

A substrate of 0.1 M KNO_3 in 0.1 M phosphate buffer with 0.1% Triton X was prepared (pH 7.0 after adjusting with NaOH). 200 mg (fresh mass) of 5 mm diameter leaf discs or 5 mm long sections of root were placed into test tubes, and 5 ml of the substrate added. The substrate was vacuum infiltrated into the tissues using a high volume syringe, after which the test tubes placed in a water bath at 30°C in darkness for 30 min. During this time the enzyme reduces some of the NO_3 in the substrate to NO_2. The boiling tubes were then placed in a water bath at 80°C for 10 min to halt the reaction. 1 ml of substrate was added to 2 ml of a combined reagent of sulfanilamide and NEDA. The reagent was made up of 1 part sulfanilamide reagent (2 g sulfanilamide (BDH Merck, UK) + 5 ml concentrated HCl diluted to 100 ml with distilled water) and 1/10 part NEDA (N-1-Napthyl)-ethylene-diamine-dihydrochloride) reagent (0.07 g NEDA (BDH Merck, UK) in 50 ml distilled water). The combined reagent undergoes a red colour reaction with nitrite. The absorbance of the solution was read using a spectrophotometer at 540 nm, using 1 ml of buffered substrate mixed with 2 ml of combined reagent as a blank. A calibration curve of was made using $NaNO_2$ (BDH Merck, UK) as a standard. An example of a standard curve is given in Figure 2.14 ($y = 0.0892x$, $r^2 = 0.9857$, $p < 0.001$). The amount of nitrite present is calculated by:

absorbance x slope = μmol NO_2 ml^{-1} substrate

The value obtained is multiplied by 5 to give the amount of NO_2 produced by the tissues in 5 ml of substrate, then divided by the fresh weight (g) of the samples, and multiplied by 2 to give the amount of NO_2 produced per hour. Nitrate reductase activity is expressed in μmol NO_2 g^{-1} fw h^{-1}.

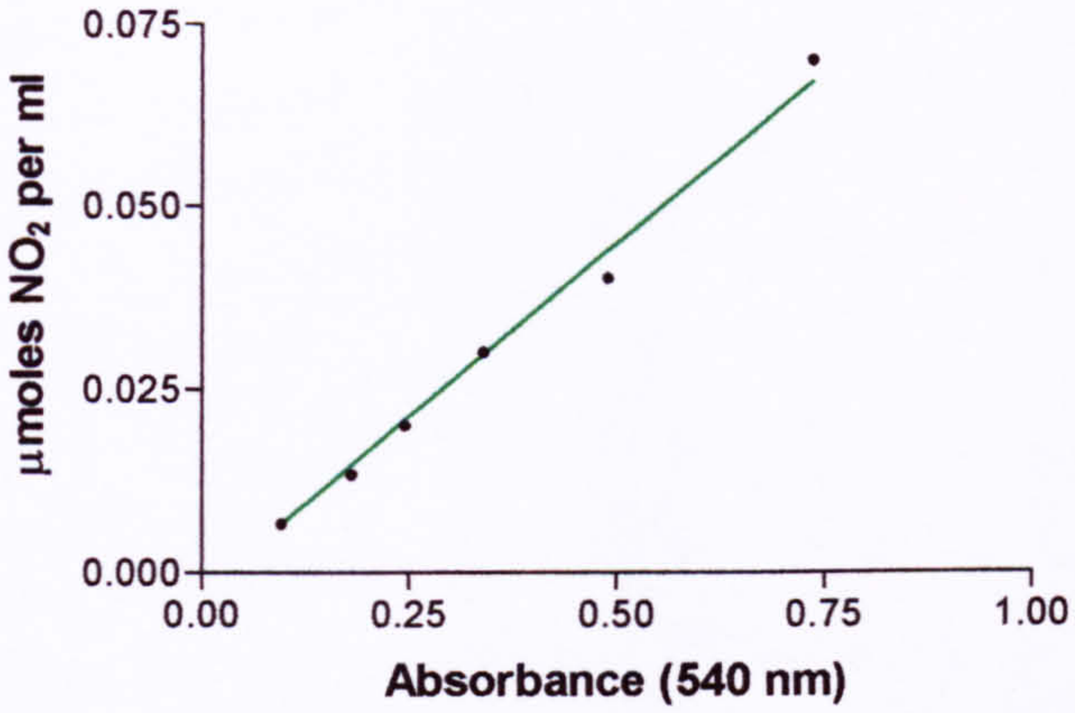


Figure 2.14 Calibration curve of $NaNO_2$ standards ($y = 0.0892x$, $r^2 = 0.9857$, $p < 0.001$).

2.2.10 Measurement of NO_2 concentrations

NO_2 concentrations were measured using NO_2 passive diffusion tubes (Atkins *et al.*, 1978). The NO_2 tubes were purchased from Gradco International Ltd. (Winchester, UK). The acrylic tubes of 71 mm length have an inner diameter of 9.5 mm and an outer diameter of 12.7 mm. Tubes were washed with detergent, then with 0.5% hydrochloric acid (HCl; BDH Merck, Pool, Dorset, UK). They were then rinsed with distilled water and air dried. Stainless steel fine mesh gauze discs of 12 mm diameter were cleaned using 0.5% HCl, rinsed with distilled water and air dried. Discs were then dipped into a solution of 50% triethanolamine (Sigma-Aldrich, Pool, Dorset, UK) and 50% acetone (Sigma-Aldrich, UK) and placed on absorbent paper to dry. Two discs were stacked in the lid at one end of each diffusion tube. A lid was placed at the opposite end of the tube until the tubes were placed in the field, when they were removed to allow ambient air to diffuse in. Atmospheric NO_2 diffuses into the tubes and readily reacts with the triethanolamine on the wire gauze to form nitrite. Tubes were exposed for 12-14 days, then collected and returned to the laboratory for analysis. Two sealed tubes were retained to be used later as controls in the calibration.

A combined reagent of sulfanilamide and NEDA (as described in Section 2.2.9) was prepared. The protective lids of the NO_2 diffusion tubes were removed, and 3 ml of combined reagent pipetted into each tube. The tubes were shaken gently and left for 25 minutes. The reagent reacts with any nitrite present on the wire mesh to give a pink colour reaction.

The absorbance of the solution was read using a spectrophotometer at 540 nm, using the reagent as a blank, and using the control tubes to check for contamination during preparation of the tubes. A calibration curve of was made using $NaNO_2$ (BDH Merck, UK) as a standard. An example of a standard curve is given in Figure 2.15 ($y = 1.0175x$, $r^2 = 0.9985$, $p < 0.001$). The amount of nitrite present on the discs is converted to ppb NO_2 per hour using the following equation:

$$NO_2 \text{ (ppb)} = \frac{\text{absorbance x slope x } 10^5}{2.3 \text{ x exposure time (h)}}$$

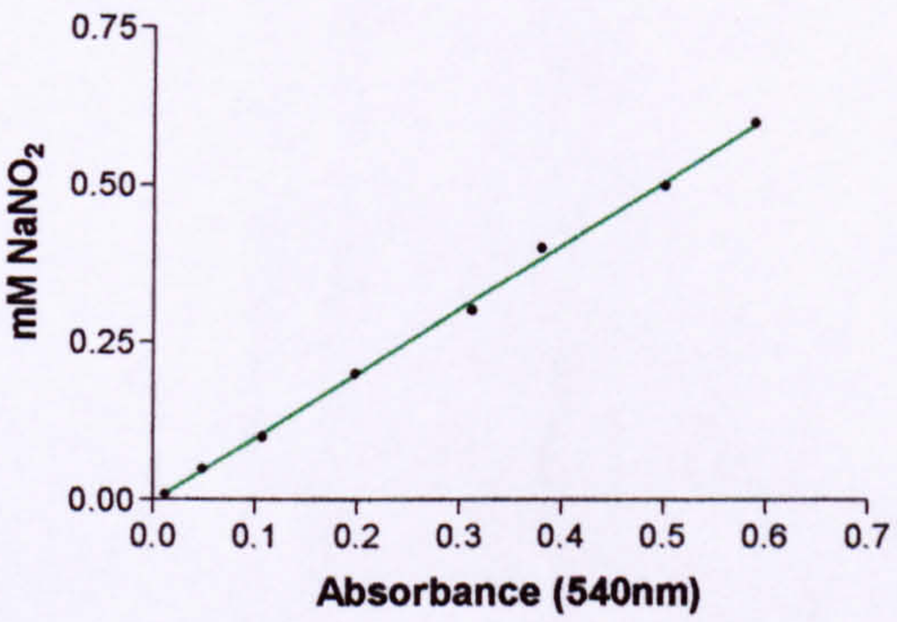


Figure 2.15 Standard curve for the conversion of nitrite absorbance values.
($y = 1.0175x$, $r^2 = 0.9985$, $p < 0.001$).

2.2.11 Overview of plant measurements

Table 2.4 shows the time course of measurements taken in each year of Solardome experiments.

Table 2.4 Timing of exhaust gas exposure and of physiological measurements made on plants in each year of experiments

MONTH	**2000**	**2001**	**2002**
April		Start of Exposure (22/04/01) **day 1**	
May			Start of Exposure (27/05/02) **day 1**
June	Start of Exposure (03/06/00) **day 1**	Contact angles (24/06/01) **day 64** Stomatal conductance (27/06/01) **day 67**	Start of N addition (06/06/02) **day 11** Stomatal conductance (17/06/02) **day 22**
July	Stomatal conductance (08/07/00 – 18/07/00) **days 36-46**	Start pf drought (08/07/01) **day 78** Water potentials End of drought (timing depending on species)	Stomatal conductance and photosynthesis (16/07/02) **day 51**
August	Leaf drying rates (02/08/00 – 04/08/00) **days 61-63** Contact angles (08/08/00 – 11/08/00) **days 67-70**	Stomatal conductance (25/08/01) **day 126** Leaf samples collected for carbon isotope discrimination (26/08/01) **day 127**	Stomatal conductance and photosynthesis (13/08/02) **day 79** Chlorophyll fluorescence (13/08/02) **day 79**
September			End of N addition (12/09/02) **day 109** Leaf retention (24/09/02) **day 121** Leaf senescence by colour (24/09/02) **day 121** Leaf senescence by chlorophyll fluorescence (25/09/02) **day 122** Leaves collected for nitrogen analysis (25/09/02) **day 122** Nitrate reductase activity (26/09/02) **day 123**
October			Leaves collected for SEM observations 02/10/02 **day 129**

Chapter 3: Initial Screening Experiment and Selection of Study Species

3.1 Introduction

The responses of plants to urban pollution have been fairly well studied in the field (e.g. Huttunen and Ruonala, 1986, cited in Huttunen, 1994; Kammerbauer *et al.*, 1987; Pal *et al.*, 2002). Only two other fumigation studies have been carried out under controlled environmental conditions, both using Norway spruce. Sauter *et al.* (1987) studied alterations in epicuticular waxes, and Viskari *et al.* (2000b) also looked at the effects on waxes as well as stomatal diffusive resistance. Both studies found surface waxes to be significantly degraded by the pollution mixtures, and Viskari *et al.* (2000b) found stomatal resistance was reduced (i.e. stomata lost their ability to close) during the night.

In the present study, nineteen species of broadleaved trees and shrubs commonly planted in cities were screened for sensitivity to urban pollution mixtures in an attempt to identify species exhibiting responses to this type of pollution. A range of measurements were made on growth, stomatal responses, rates of water loss, and surface characteristics. This initial screening study allowed the fumigation system to be tested and refined, and was useful in selecting species for further experiments.

3.2 Materials and Methods

3.2.1 Plant material.

One-year old plants were purchased from Cheviot Trees (Berwick upon Tweed, Northumberland). Twenty plants of each species were allocated to the four Solardomes, with five replicates per dome. Pots and soil used are described in Section 2.2.1. The plants were placed in the Solardomes on 3rd June 2000. This was later than intended, due to a delay in getting the fumigation system running. Therefore the plants had already gone through some of their growing phase before being assigned to the treatments.

Species:

Acer pseudoplatanus L. (sycamore)

Buddleja davidii Franchet (butterfly bush)

Cornus sanguinea L. (dogwood)

Euonymus japonicus L. fil.

Fagus sylvatica L. (common beech)

Fraxinus excelsior L. (common ash)

Hebe carnosula Hook. f.

Hydrangea macrophylla "Lacecap"

Hydrangea macrophylla "Pink"

Hypericum androsaemum L. (tutsan)

Ligustrum ovalifolium Hassk. (privet)

Pittosporum tenuifolium Gaertn.

Quercus robur L. (pedunculate oak)

Rosa rubiginosa L. (sweet brier)

Salix caprea L. (pussy willow)

Sambucus nigra L. (common elder)

Sorbus aria L. (whitebeam)

Spiraea salicifolia L.

Viburnum davidii Franchet

3.2.2 Stomatal conductance

Conductance was measured as described in Section 2.2.2. Measurements were made on 16 species during July 2000.

3.2.3 Contact angle measurements

Droplet contact angles of recently fully-expanded leaves were made on 12 species representing a range of leaf surface types during August 2000, as described in Section 2.2.5.

3.2.4 Leaf drying rates

Measurements of leaf drying rates were taken in August 2000, as described in Section 2.2.6.

3.2.5 Growth (percentage increase in height)

Plant height (H) was measured in June 2000, and again in September 2000. The percentage increase in height between June (t_1) and September (t_2) was calculated by:

$$\% \text{ increase in height} = \left[\frac{H(t_2)/H(t_1)}{H(t_2)} \right] \times 100$$

3.2.6 Statistical analysis

Statistics were performed using a standard SPSS statistics package (SPSS Inc., Chicago, USA). Data were checked for normal distribution and homogeneity of variance, then tested for chamber effects within treatments using ANOVA with Duncan's multiple range test. No chamber effects were found. For stomatal conductance and relative water content, data were subjected to two-factor repeated measures ANOVAs. Factors were treatments (CFA and exhaust gas pollution), and time period. For other parameters, oneway ANOVAs were used to compare plants in exhaust gas-polluted air and CFA. For plant height, percentage data were arcsine transformed. Actual values for final plant height were subjected to univariate ANOVA, using original height as a covariate in order to test for effects of pollution (the fixed factor).

3.3 Results

Responses to all the parameters measured are summarised in Table 3.1.

Table 3.1 Responses of 20 species of trees and shrubs to urban pollution mixtures. No effect of exhaust gas pollution (0); decrease in parameter in exhaust gas-polluted air compared with CFA (↓); increase in parameter in exhaust gas-polluted air compared with CFA (↑).

Species	growth	G_s	contact angles	RWC
Acer pseudoplatanus	0	0		
Buddleja davidii	0	↓		
Cornus sanguinea *	0	0	0	0
Euonymus japonicus	0	0		
Fagus sylvatica	0	0		
Fraxinus excelsior	0	0	0	
Hebe carnosula	0		0	0
Hydrangea macrophylla L *	0	↑	0	
Hydrangea macrophylla P *	0	0	0	0
Hypericum androsaemum	0	0	0	
Ligustrum ovalifolium *	0	0	↑	
Pittosporum tenuifolium	0	0	0	0
Quercus robur *	0		↓	
Rosa rubiginosa	0	0	0	0
Salix caprea	0	0		0
Sambucus nigra	0	0		
Sorbus aria	0	↓		
Spiraea salicifolia	0		0	
Viburnum davidii	0	↓	0	0

* Species selected for further study

Figure 3.1 Percentage increase in height (mean ± SE) between June and September 2000 in a variety of tree/shrub species in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data have been arcsine transformed. n=10.

3.3.1 Growth

There were no significant effects of exhaust gas pollution on percentage increase in plant height between June and September 2000 in any of the species studied (Figure 3.1). Univariate ANOVA revealed a significant effect of original height on final height, and no significant influence of exhaust gas pollution.

3.3.2 Stomatal conductance

Stomatal conductance of plants in exhaust gas-polluted air and CFA are shown in figures 3.2 - 3.6. Repeated measures ANOVAs for stomatal conductance in all species measured are given in Appendices 1-16. Of the species that exhibited a stomatal response (four out of 16 species), the majority exhibited lower stomatal conductance in exhaust gas-polluted air compared with CFA. In *Buddleja davidii* and *Sorbus aria*, exhaust gas pollution had an overall effect of decreasing stomatal conductance (repeated measures ANOVAs; $p=0.050$ and 0.023, respectively; Appendices 2 and 15) compared with plants in CFA. *Viburnum davidii* exhibited lower conductance in polluted conditions only at certain times during the day (13:00). This effect was verging on significance (oneway ANOVA; $p=0.057$). In *Hydrangea macrophylla* "lacecap", the pollution mixture had the opposite overall effect of increasing stomatal conductance compared with clean air controls (repeated measures ANOVA; $p=0.005$; Appendix 7).

3.3.3 Contact angle measurements

Figure 3.7 shows the angles of contact of water droplets on leaves of 12 species. Of the 12 species on which measurements were made, two exhibited differences between exhaust gas-polluted air and CFA. In *Quercus robur*, leaves in exhaust gas-polluted air had significantly (oneway ANOVA; $p<0.001$) lower angles of contact compared with clean air controls. *Cornus sanguinea* showed the opposite situation, with leaves in exhaust gas-polluted air having higher contact angles compared with CFA (oneway ANOVA; $p<0.05$).

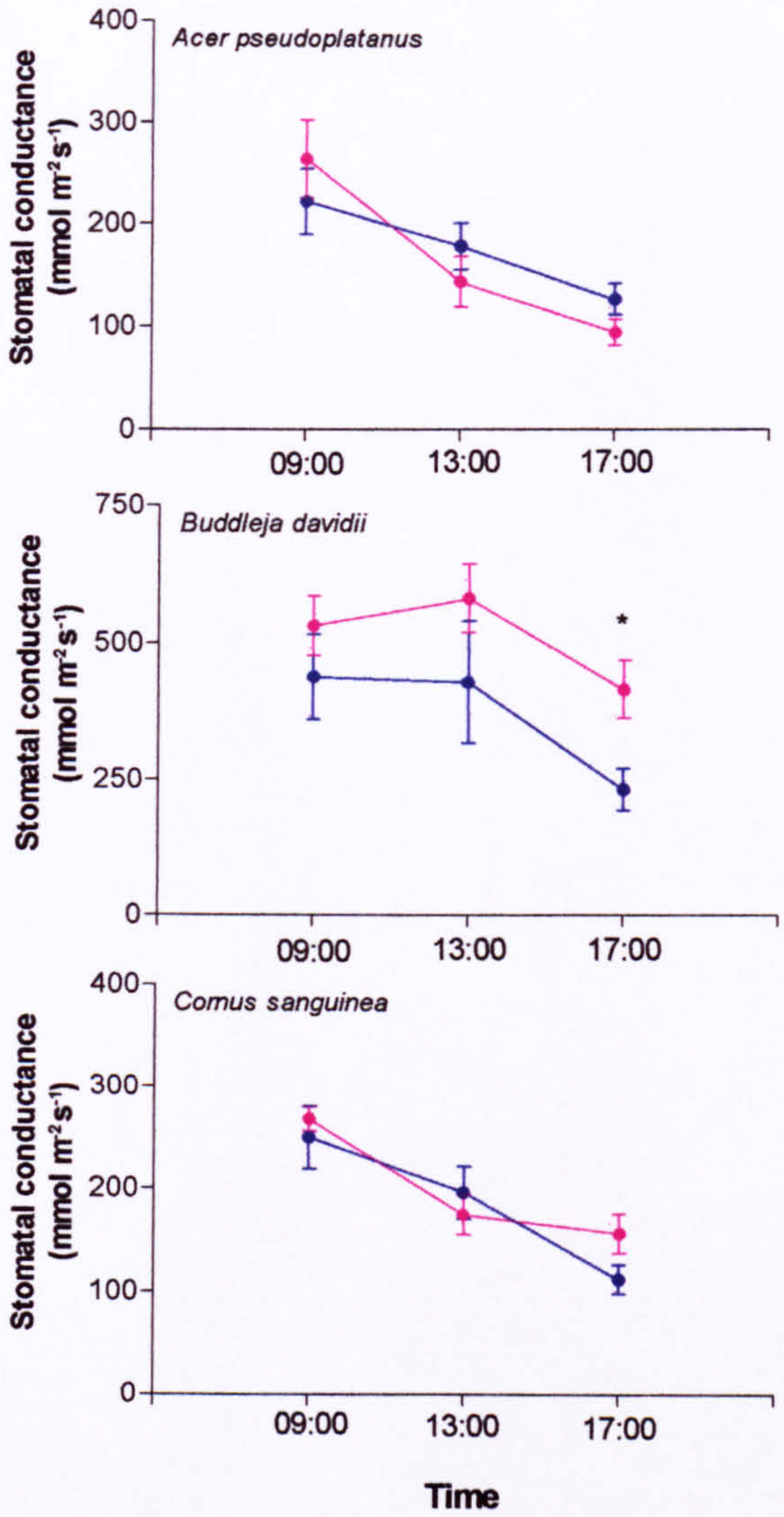


Figure 3.2 Stomatal conductance (mean ± SE) in three tree/shrub species grown in CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). Asterisks denote the probability of difference (oneway ANOVA) between CF and exhaust gas-polluted plants (* $p<0.05$). n=10.

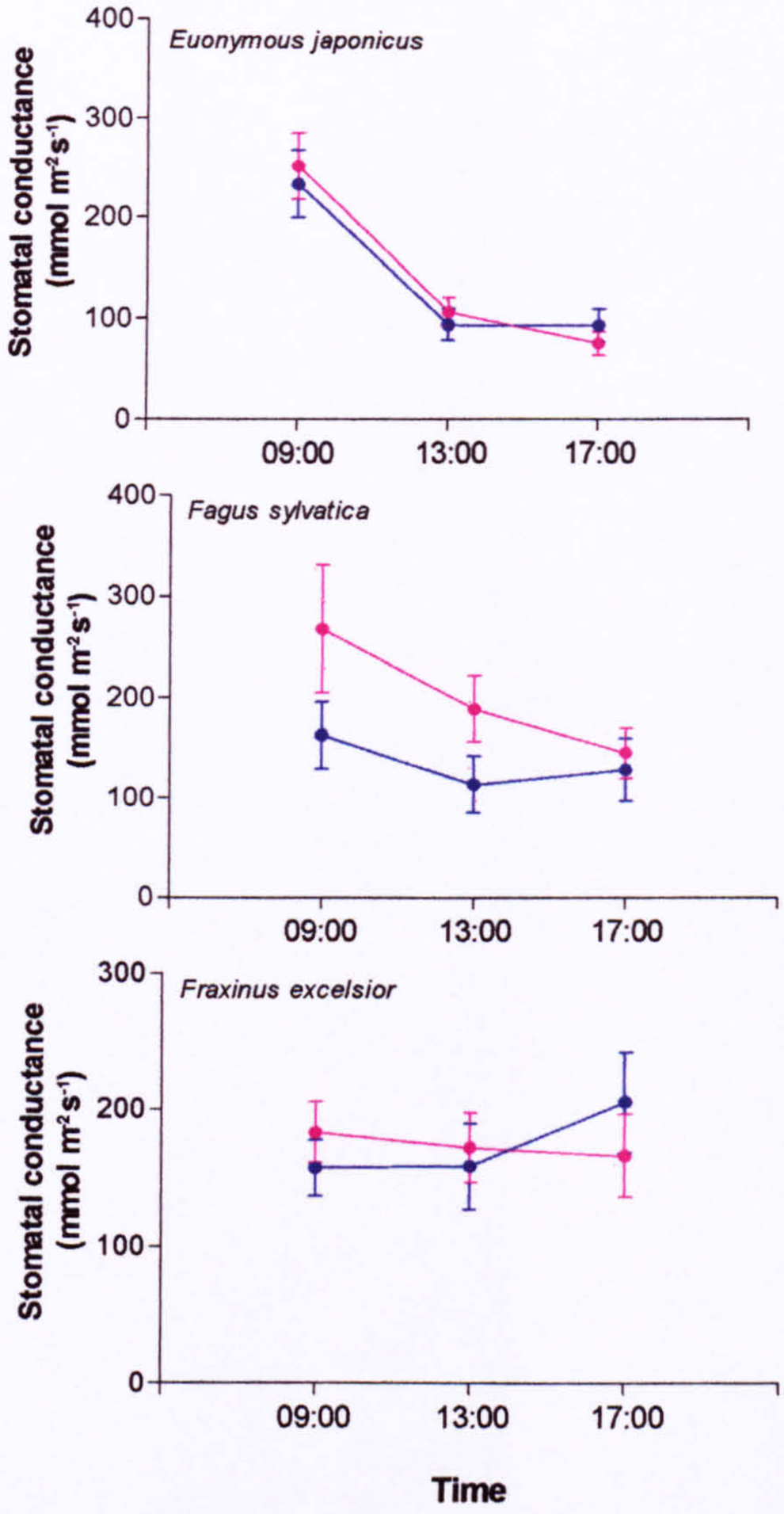


Figure 3.3 Stomatal conductance (mean ± SE) in three tree/shrub species grown in CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x).

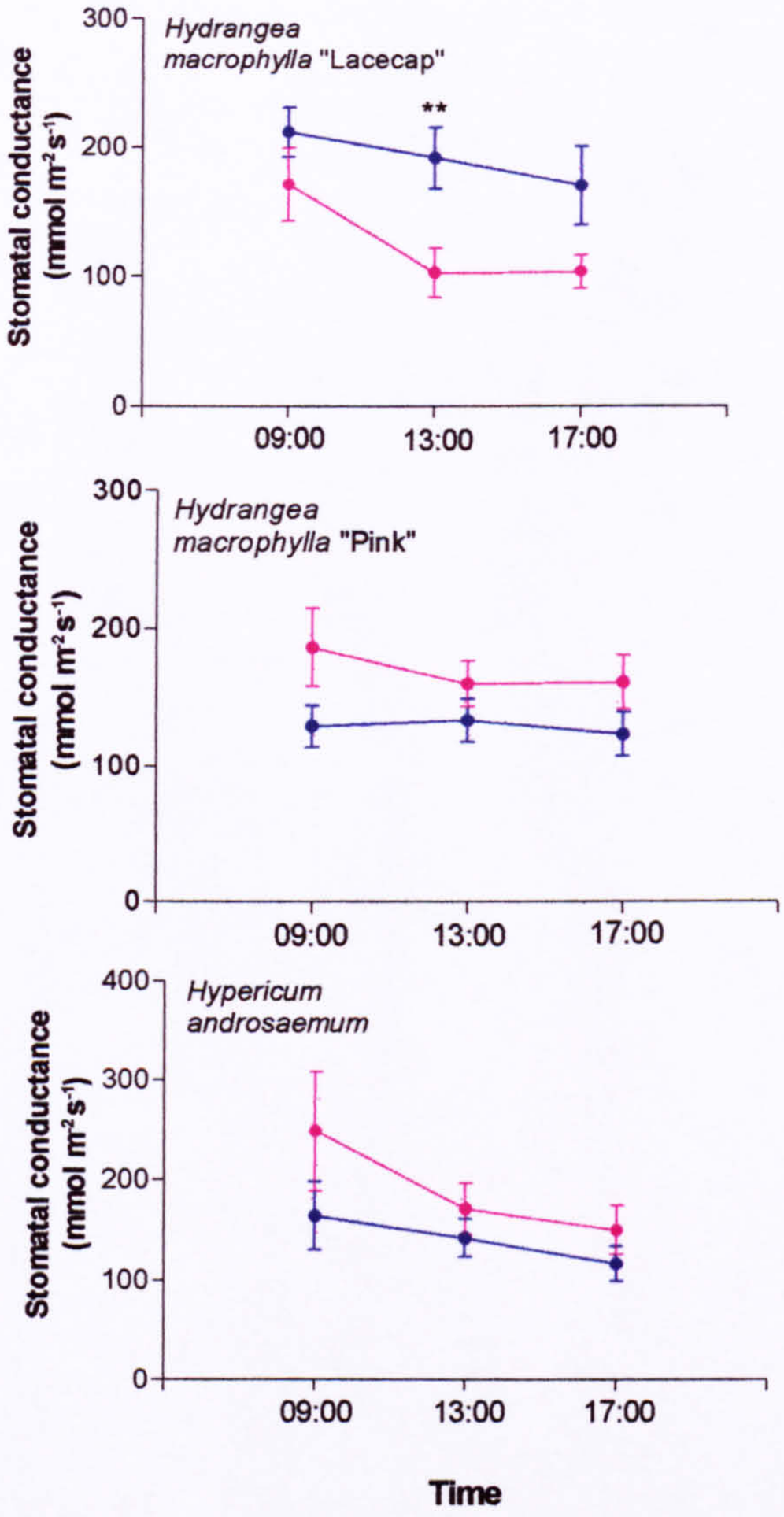


Figure 3.4 Stomatal conductance (mean ± SE) in three tree/shrub species grown in CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). A repeated measures ANOVA showed a significant increase in stomatal conductance in *Hydrangea macrophylla* "Lacecap" under exhaust gas pollution. Asterisks denote the probability of difference (oneway ANOVA) between CF and exhaust gas-polluted plants (** $p<0.01$). n=10.

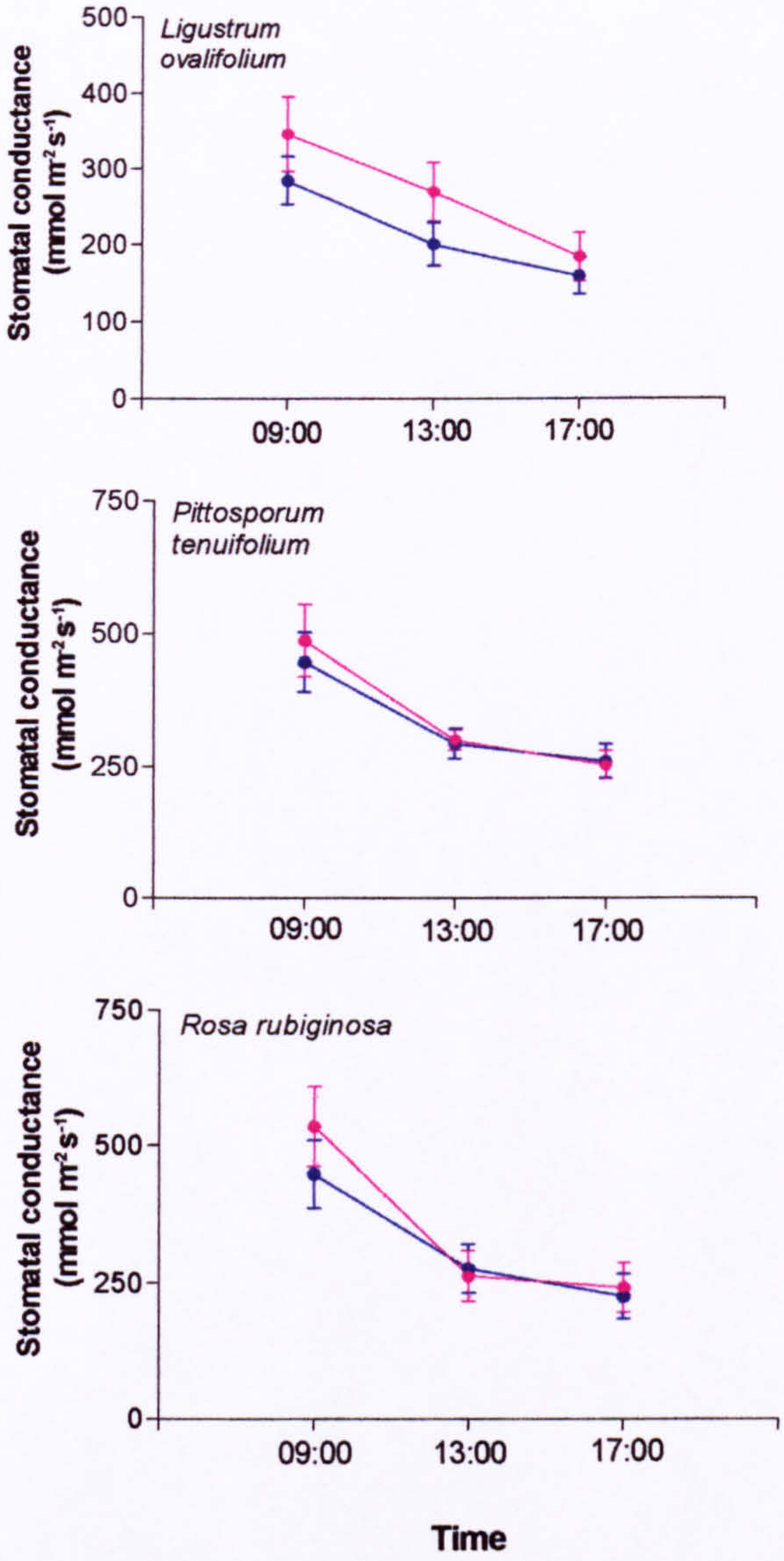


Figure 3.5 Stomatal conductance (mean ± SE) in three tree/shrub species grown in CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

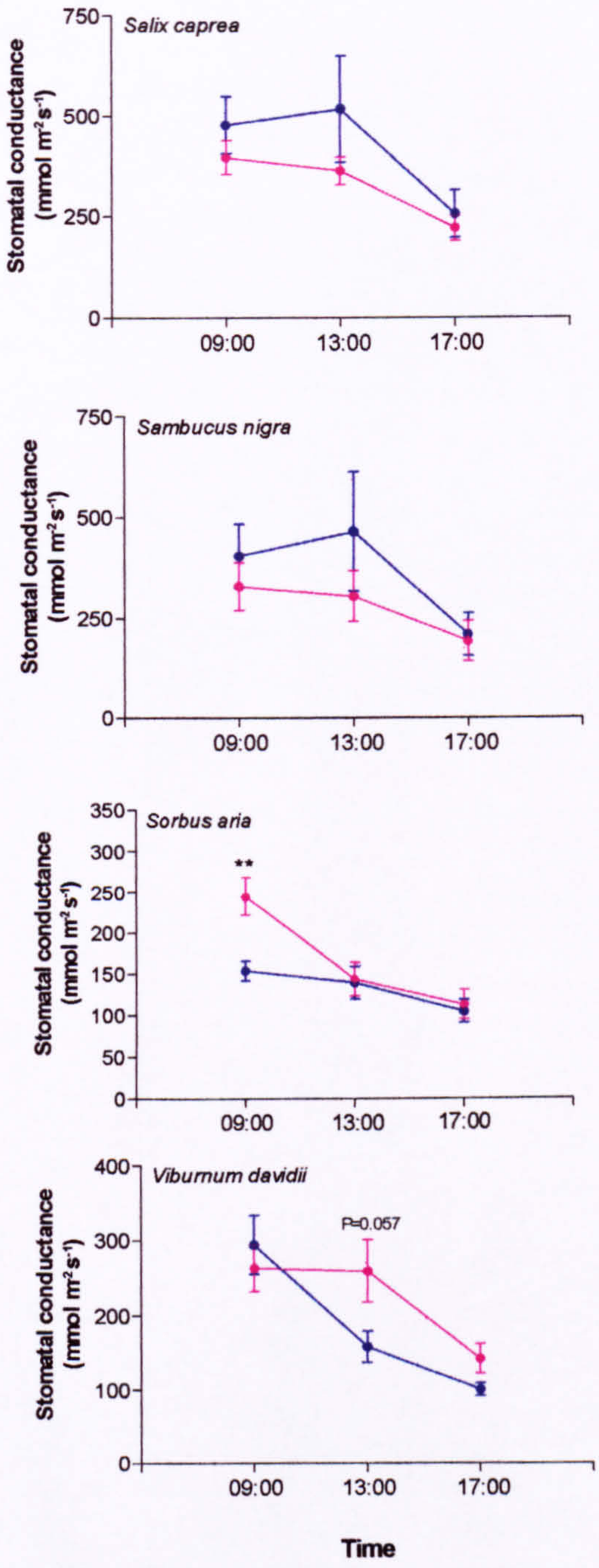


Figure 3.6 Stomatal conductance (mean ± SE) in four tree/shrub species grown in CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). A repeated measures ANOVA showed a significant decrease in stomatal conductance in *Sorbus* aria. Asterisks denote the probability of difference (oneway ANOVA) between CF and exhaust gas-polluted plants (** $p<0.01$). n=10.

Figure 3.7 Angles of contact of water droplets (mean ± SE) on leaves of a range of tree/shrub species in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Asterisks denote the probability of difference between CF and exhaust gas-polluted plants (* $p<0.05$; *** $p<0.001$). n=10.

3.3.4 Leaf drying rates

Rates of water loss from excised leaves, as determined by RWC over time, are given in Figures 3.8 – 3.14. None of the species exhibited differences in leaf drying rates between treatments (tested by repeated measures ANOVAs; Appendices 17-23).

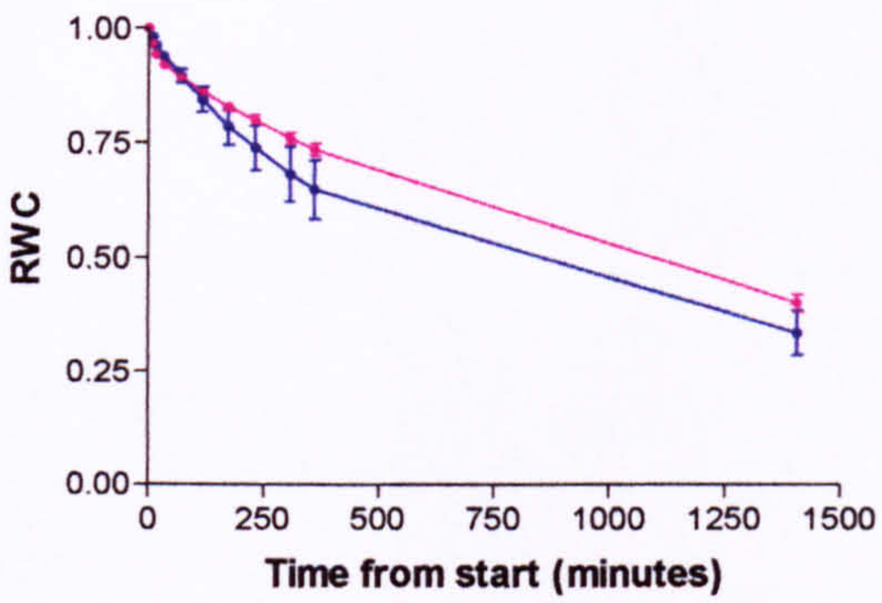


Figure 3.8 Rate of loss of water (mean ± SE) from *Cornus sanguinea* leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

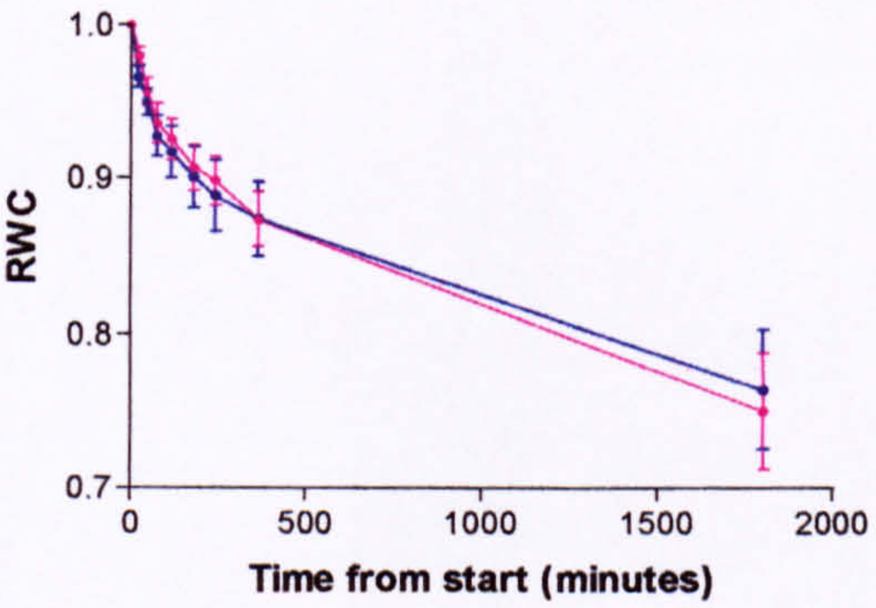


Figure 3.9 Rate of loss of water (mean ± SE) from *Hebe carnosula* leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

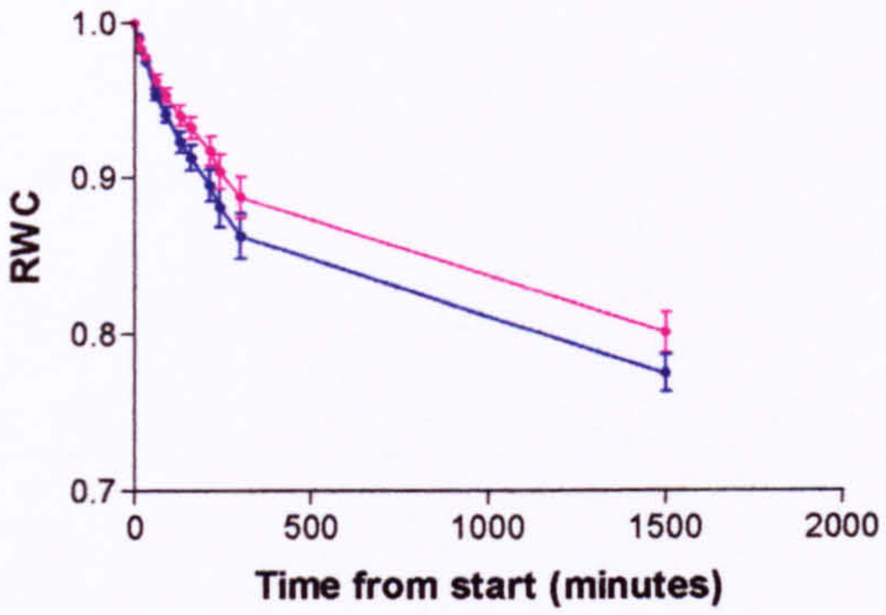


Figure 3.10 Rate of loss of water (mean ± SE) from *Hydrangea macrophylla* "Pink" leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

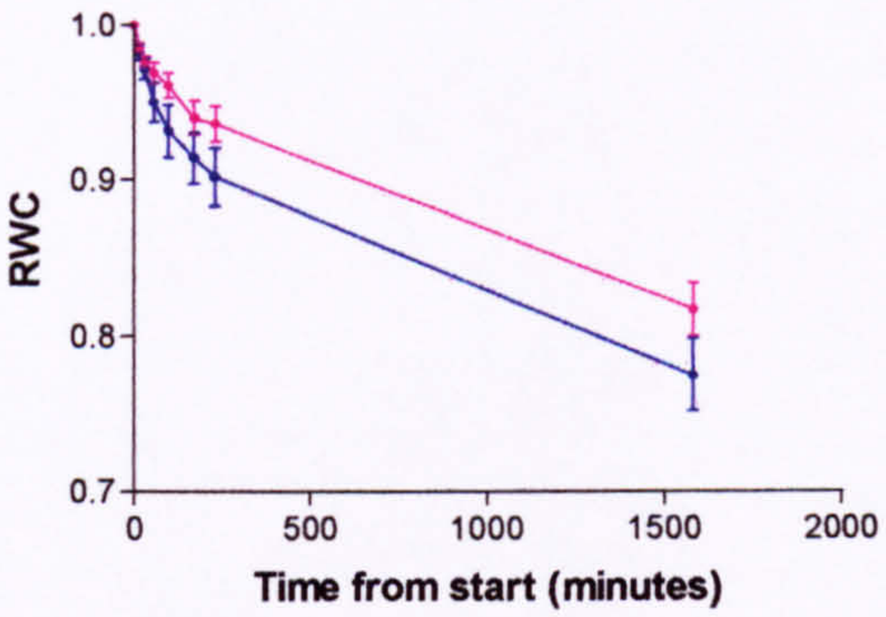


Figure 3.11 Rate of loss of water (mean ± SE) from *Pittosporum tenuifolium* leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

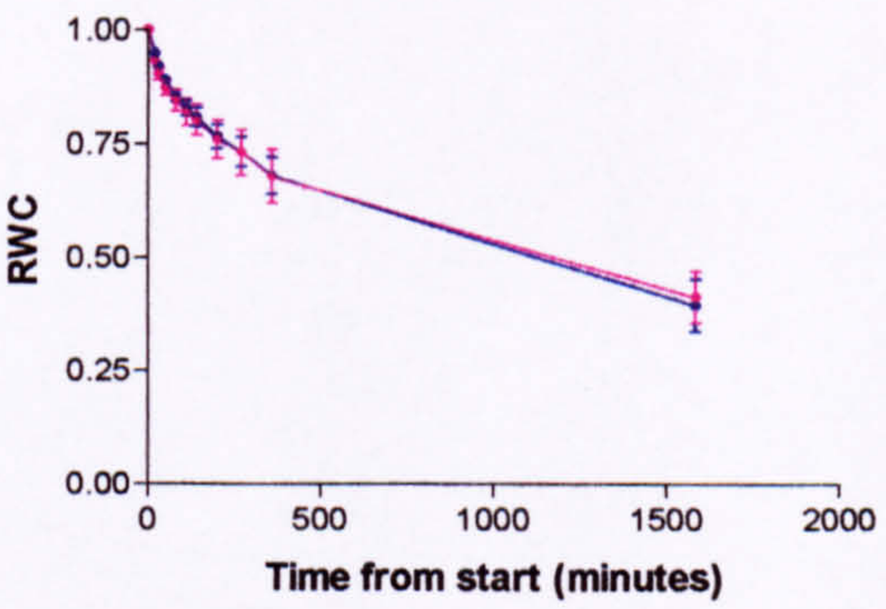


Figure 3.12 Rate of loss of water (mean ± SE) from *Rosa rubiginosa* leaves grown under CFA (●) and

exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

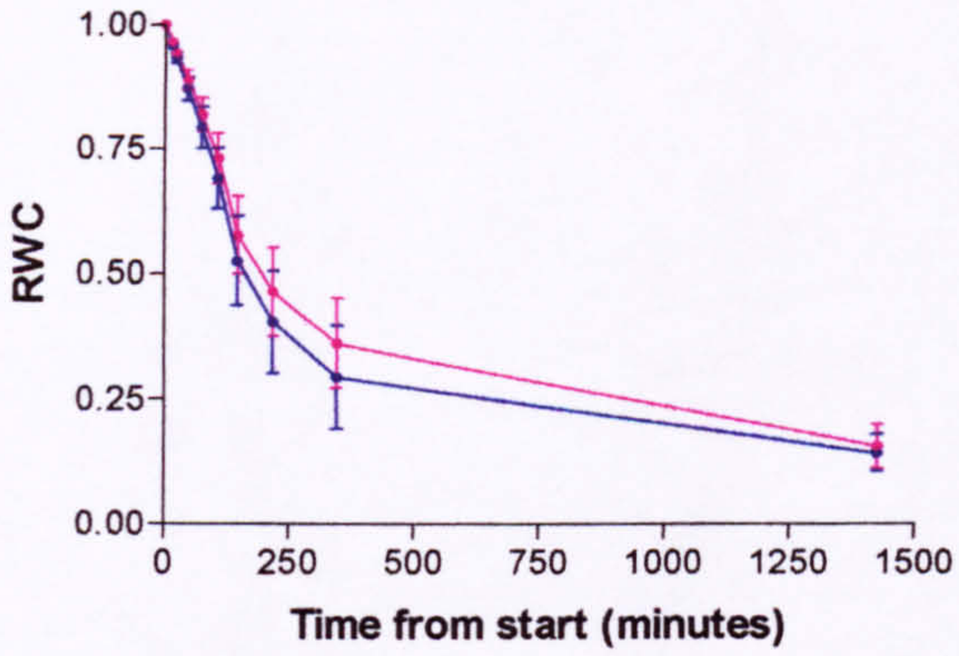


Figure 3.13 Rate of loss of water (mean ± SE) from *Salix caprea* leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

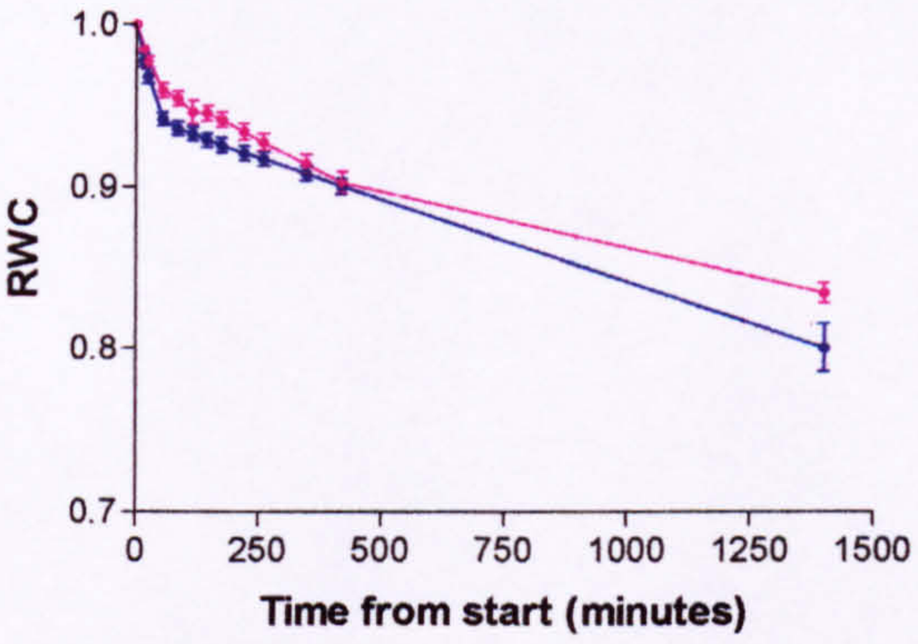


Figure 3.14 Rate of loss of water (mean ± SE) from *Viburnum davidii* leaves grown under CFA (●) and exhaust gas-polluted conditions (●; 100 ppb NO_x). n=10.

3.4 Discussion

Overall, this study revealed that urban pollution mixtures have surprisingly little effect on typical urban woody plant species, and these effects are subtle. Leaf gas exchange was altered in some species, in contrasting directions. It is possible that those species showing a stomatal closure response might be more tolerant to the pollution, since the effective pollutant dose would be reduced (Darrall, 1989). Decreased gas exchange might also be expected to have consequences for carbon

gain, but growth was not affected in any of the species studied. However, as mentioned earlier, the late start to the experiment meant that the early part of the growing season was missed.

Stomatal conductance and the condition of leaf surface waxes also have potential consequences for water loss. However, water loss from excised leaves was not affected by urban pollution mixtures in the present study. Using contact angle measurements, the hydrophobic properties of leaf surface waxes were found to be affected by the pollution in two of the species studied (*Quercus* and *Ligustrum*). Previous studies (mainly using conifers) have shown using SEM observations that epicuticular waxes are structurally degraded by the presence of a variety of air pollutants (e.g. Trimble *et al.*, 1982; Sauter *et al.*, 1987; Viskari *et al.*, 2000b).

Species were selected for further study on the basis of their responses to the exhaust gas pollution. The species chosen on the basis of stomatal response was *Hydrangea macrophylla* "Lacecap", which exhibited higher stomatal conductance in exhaust gas-polluted air compared with CFA, *Ligustrum ovalifolium* and *Quercus robur* were selected because their surface characteristics, as determined by droplet contact angles, appeared to be affected by the pollution mixtures. In *Quercus robur*, leaves had significantly lower droplet contact angles in polluted air compared with CFA, and in *Ligustrum ovalifolium*, the opposite response was found. *Cornus sanguinea* and *Hydrangea macrophylla* "Pink" had shown few responses to the exhaust gas mixture, and were selected for their apparent resistance to urban pollution.

Chapter 4: The Effects of Urban Pollution Mixtures on Response to Water Stress

4.1 Introduction

4.1.1 Influence of air pollutants on gas exchange and photosynthesis

The influence of air pollutants on stomatal behavior varies between pollutants, with pollution concentrations, plant species, plant age and growing conditions, so that it is difficult to summarize or to make generalizations on these responses. Under normal conditions, stomatal opening is considered to strike a balance between carbon acquisition and water loss, so that any perturbation in stomatal control could interfere with either or both of these important processes (Mansfield and Lucas, 1996).

Perturbations in stomatal behaviour have been observed in fumigations with combinations of NO_2, SO_2 and O_3 (e.g. Schenone *et al.*, 1994) and NO_2 and SO_2 (Neighbour *et al.*, 1988). Increases in stomatal conductance were observed in *Euonymus japonica* plants fumigated with NO_2 (Natori and Totsuka, 1984). Some decreases in stomatal conductance have also been reported in response to NO_2 (Darrall, 1989). Particulates, also an important component of urban pollution, have been found to increase stomatal conductance, probably by causing mechanical obstructions of stomatal pores, preventing complete closure (Mansfield and Mejernik, 1970; Ricks and Williams, 1974). Less work has been carried out using complete mixtures of urban pollutants. On exposure of Norway spruce to vehicle pollution at the roadside, Kammerbauer *et al.* (1987) found a suppression of stomatal conductance. In fumigations of spruce with vehicle exhaust gasses, Viskari *et al.* (2000b) found no effect on stomatal resistance during the daytime, but reduced resistance during the night.

Where a stomatal opening response occurs, the effective pollutant dose entering the mesophyll will be increased. Loss of the regulating capabilities of the stomata is thought to be due to damage to cells surrounding the guard cells. Electron microscope studies of leaves exposed to mixtures of SO_2 + NO_2 (Neighbour *et al.*, 1988) have shown selective damage to epidermal cells surrounding stomata

that exhibited abnormally wide apertures. This may represent a failure of the hydrostatic mechanism of stomatal closure, whereby turgor pressure generated in the guard cells pushes against the resistance offered by the turgor of surrounding cells (Black and Black, 1979; Mansfield and Lucas, 1996).

Where stomata close in response to pollution, this may not be a direct reaction to the presence of air pollutants. There are examples of stomatal closure occurring only after reductions in photosynthetic rates, in which case the stomata are responding to lower CO_2 uptake due to an inhibition of photoassimilation (reviewed in Robinson *et al.*, 1998). This might explain the decrease in water use efficiency which sometimes accompanies pollution-induced partial stomatal closure (Shan *et al.*, 1996). A side effect of this closure is a reduction in pollutant uptake, which should protect metabolizing tissues from injury (Darrall, 1989). This also shows that there may be a lag time in stomatal response to pollutants, with the activation of guard cell turgor requiring active biochemical reactions (Musselman and Minnick, 2000).

Disturbances of stomatal function observed in response to air pollutants can be the result, or the cause, of alterations in photosynthesis. Several studies have looked at the effects on photosynthesis of NO_x, alone and in combination with other pollutants. NO_2 has greater-than-additive effects in combination with SO_2 (White *et al.*, 1974) and O_3 (Furukawa *et al.*, 1984), but where gases are applied singly, photosynthesis appears to be less sensitive to NO_2 than to other pollutants. There is evidence that NO has a greater inhibitory effect on photosynthesis compared with NO_2 (Saxe, 1986). This could be important in the urban environment, since NO is more abundant than NO_2 close to sources of vehicle emissions. Studies of the effects of NO_x have given varied results, with observations of reductions, increases or no effects on photosynthetic rates (Wellburn, 1990). Plant species differ in their responses, but also the direction of changes in photosynthesis can vary depending on growing conditions and NO_2

concentrations, with lower concentrations often increasing net photosynthesis and high concentrations suppressing it (Wellburn, 1990).

Few studies have investigated the effects of realistic mixtures of urban pollutants on photosynthesis. Kammerbauer *et al.* (1987) found a 31% decrease in net assimilation rates in Norway spruce plants exposed to pollution along a highway compared with those at a control site. This was shown to be the result of a combination of decreased stomatal conductance and photosynthetic metabolism.

In exposures of plants to NO_x, there are no consistent patterns for alterations in respiration. Both enhanced and suppressed respiration have been reported for different species (Darrall, 1989; Wellburn, 1990). Enhanced rates of respiration may aid in repair and detoxification mechanisms (Darrall, 1989). Pollutants could influence respiration through alterations in amino acids, ammonium and nitrite which might be expected to have secondary effects on mitochondrial enzymes and levels of ATP (Wellburn, 1990).

4.1.2 Interactions between air pollutants and water stress

Features of the urban environment such as higher temperatures and vapour pressure deficits plus rapid run-off of rain and compacted soils with poor water permeability mean that plants growing in urban situations often encounter conditions of low water availability. Water stress can modify the effects of air pollution, and vice versa. For example, partial stomatal closure brought about by drought can have the side effect of reducing pollutant flux into the plant (e.g. Tingey and Hogsett, 1985). Conversely, O_3 and other pollutants can themselves cause partial stomatal closure, with the effect of reducing transpirational water loss and ameliorating the effects of drought stress (Mills, 2002). Some crop plants have been shown to be less sensitive to O_3 under conditions of drought (e.g. Temple *et al.*, 1998), but in soybean, the combination of drought and O_3 gave greater-than-additive effects on yield reduction (Heggestad *et al.*, 1985).

In some trees, drought-induced stomatal closure is impaired by O_3 (e.g. Pearson and Mansfield, 1993). MaierMaercker and Koch (1991) found poor control of water balance when conditions of decreasing atmospheric humidity were imposed on *Picea abies* twigs following fumigation with O_3. In beech, trees exposed to O_3 failed to increase their stomatal resistance in response to water stress (Pearson and Mansfield, 1993). Less work has been carried out on interactions of drought stress with pollutants other than O_3. Lucas (1990) found that Timothy grass exposed to combinations of SO_2 and NO_2 exhibited increased rates of water loss in polluted treatments under conditions of drought.

It has been suggested that pollution-induced epicuticular wax erosion (see chapter 6) might also contribute to impaired drought avoidance capabilities. If the permeability of the cuticle is increased, cuticular water loss would be greater, which could have consequences for the plants' ability to maintain leaf turgor (Darrall, 1989).

Apart from physiological responses, air pollutants can alter biomass partitioning between roots and shoots. In Timothy grass, Lucas (1990) found allocation to the roots was decreased in water stressed plants exposed to high concentrations (above 60 ppb of each) of mixtures of SO_2 and NO_2. In soybean, Heggestad *et al.* (1985) found that drought stress in combination with O_3 exposure also reduced root growth. If the effect of the pollutant(s) is to decrease the root : shoot ratio (R:S), this would be expected to exacerbate water stress by reducing the area of water-absorbing surfaces compared to that of transpiring surfaces. Plants with less investment in the root system are also less able to respond to a lower water table (Heggestad *et al.*, 1985).

4.1.3 Aims of this study

In the present study, the interaction between urban pollution mixtures and water stress was studied under the close to natural environmental conditions of the urban pollution Solardomes under known pollution concentrations.

4.2 Materials and methods

4.2.1 Plant material

Two-year old individuals of *Cornus sanguinea*, *Ligustrum ovalifolium*, *Hydrangea macrophylla* "Lacecap" and *Hydrangea macrophylla* "Pink" were purchased from Cheviot Trees (Berwick upon Tweed, Northumberland). Plants were potted as described in Section 2.2.1 and 12 plants of each species/variety assigned to each Solardome on 22 April 2001. Plants were trimmed to standard height at the beginning of the experiment.

4.2.2 Drought regime

Beginning on 8th July 2001, water was withheld from half the plants of each species/variety in each chamber. Plants under the imposed drought treatment were re-watered when wilting began to occur. The time taken for wilting to occur varied between the species/varieties, so that the length of the drought was different for each. Each day, well-watered control plants were given 100 ml of water per pot.

4.2.3 Stomatal conductance

Stomatal conductance to water vapour was measured for the second-eldest leaf of each plant as described in Section 2.2.2. Diel (24-hour) and diurnal (12-hour) patterns of conductance were measured after 9 weeks (on 27th June 2001) and 18 weeks (on 25th August 2001) of exposure, respectively.

4.2.4 Water status

Measurements of water potential, as described in Section 2.2.7 were taken at midday on alternate days over the course of the imposed drought, which continued until the onset of wilting. Recovery after re-watering was tracked for several days by continued water potential measurements.

4.2.5 Soil moisture

Soil water content was measured on alternate days during the course of the drought using a TK2-BASIC soil moisture probe (Delta T devices Ltd., Burwell, Cambridge, UK).

4.2.6 Stable carbon isotope discrimination

Because no equipment was available to measure stomatal conductance during the drought period, it was not possible to make comparisons between well-watered and drought stressed plants. It was decided that for species exhibiting a difference in water status between pollution treatments, the carbon isotope ratio of the leaves would be measured after the drought period, to give an integrated measure of stomatal conductance over time. This method is described in Section 2.2.8.

4.2.7 Growth

Plant height and canopy width at its widest point were measured throughout the exposure.

4.2.8 Biomass

In May 2002, the plants were harvested, separated into roots, woody stems and leaf material, and weighed. They were then dried to constant weight at 80 °C and re-weighed.

4.2.9 Statistical analysis

Statistics were performed using a standard SPSS statistics package (SPSS Inc., Chicago, USA). Data were checked for normal distribution and homogeneity of variance, then tested for chamber effects within treatments using oneway ANOVAs with Duncan's multiple range test. Few chamber effects were found, and are indicated in graph legends. Data from the different replicate chambers were pooled together. Main and interactive effects of exhaust gas pollution and drought were tested by twoway ANOVA. Any differences were further investigated using oneway ANOVAs and Duncan's multiple range test. For measurements taken over a time course, repeated measures ANOVAs were employed, factors being time and treatment(s).

4.3 Results

4.3.1 Stomatal conductance

After 9 weeks of exposure, stomatal conductance was significantly lower in *Cornus sanguinea* plants exposed to exhaust gas emissions compared with those in filtered air (repeated measure ANOVA, p=0.022; Appendix 24). The magnitude of the effect of pollution varied at different times during the day, and was most pronounced in the morning and early afternoon (Figure 4.1). Similarly in *Ligustrum ovalifolium*, conductance was significantly lower in exhaust gas-polluted plants compared with CFA plants (repeated measure ANOVA, p=0.050; Appendix 26; Figure 4.3), this effect being most noticeable at 18:00 on day 1. Conductance was not strongly related to the incident PAR. No differences in conductance were evident between treatments in either species during the night. Later in the season, 18 weeks into the exposure, the response of *Cornus sanguinea* and *Ligustrum ovalifolium* plants had not changed, with lower conductance in polluted compared with clean air during the day (repeated measures ANOVAs, Appendices 25 and 27; Figures 4.2 and 4.4), although in *Ligustrum ovalifolium* this effect was only significant at certain times during the day (14:00 and 17:00).

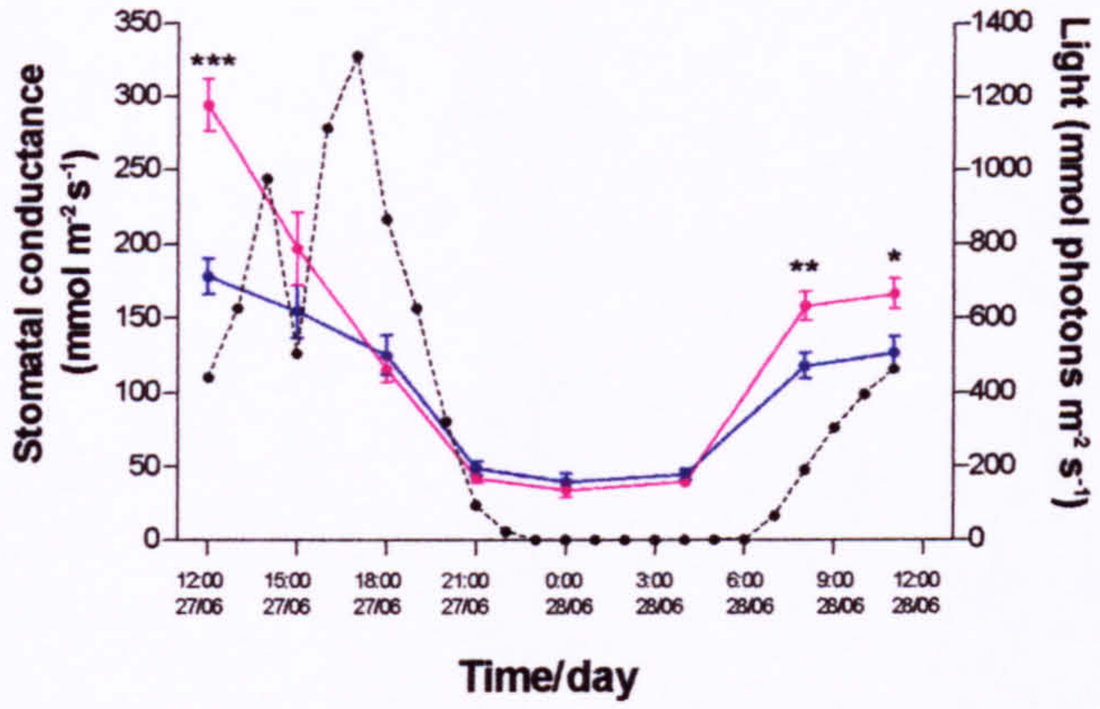


Figure 4.1 Diel pattern of stomatal conductance (mean ± SE) after 9 weeks exposure (27-28th June 01) of *Cornus sanguinea* plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant decrease in stomatal conductance in exhaust gas-polluted air compared with CFA. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted plants (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

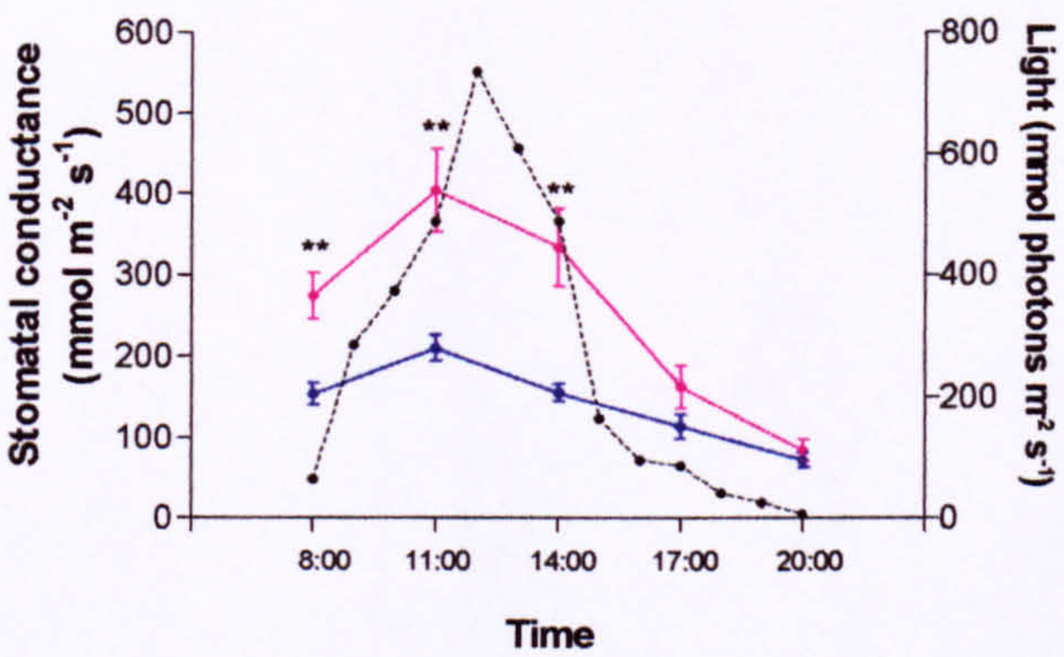


Figure 4.2 Diurnal pattern of stomatal conductance (mean ± SE) after 18 weeks exposure (25th August 01) of *Cornus sanguinea* plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant decrease in stomatal conductance in exhaust gas-polluted air compared with CFA. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted plants (** $p<0.01$).

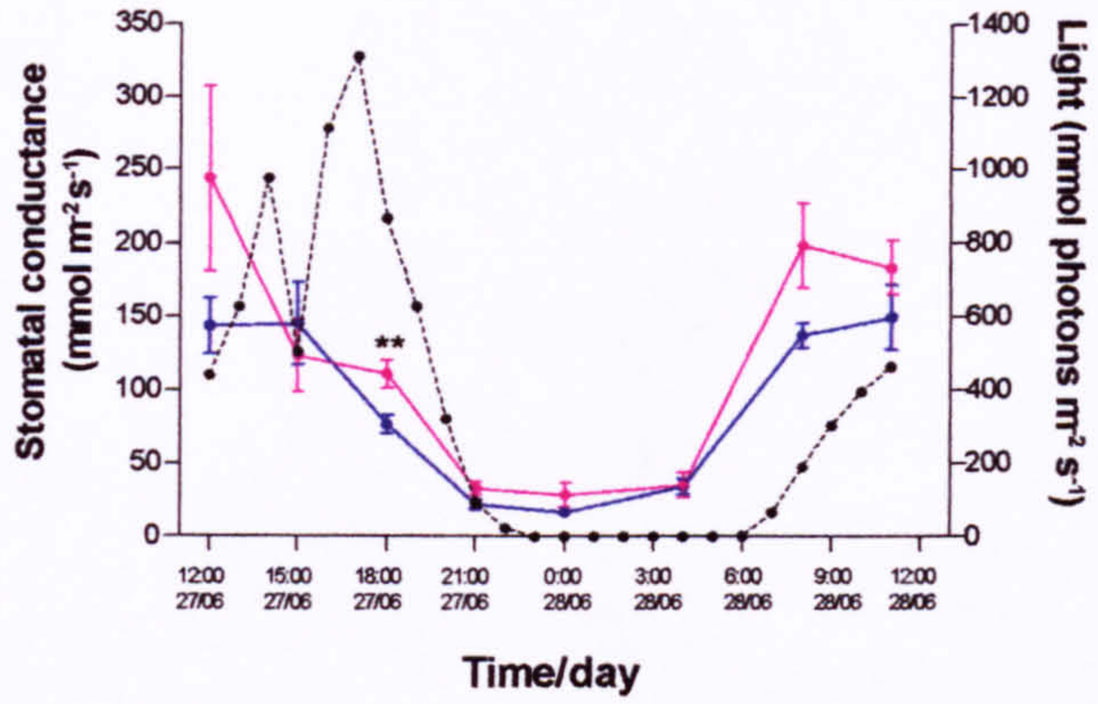


Figure 4.3 Diel pattern of stomatal conductance (mean ± SE) after 9 weeks exposure (27-28th June 01) of *Ligustrum ovalifolium* plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant decrease in stomatal conductance in exhaust gas-polluted air compared with CFA. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted groups (** $p<0.01$).

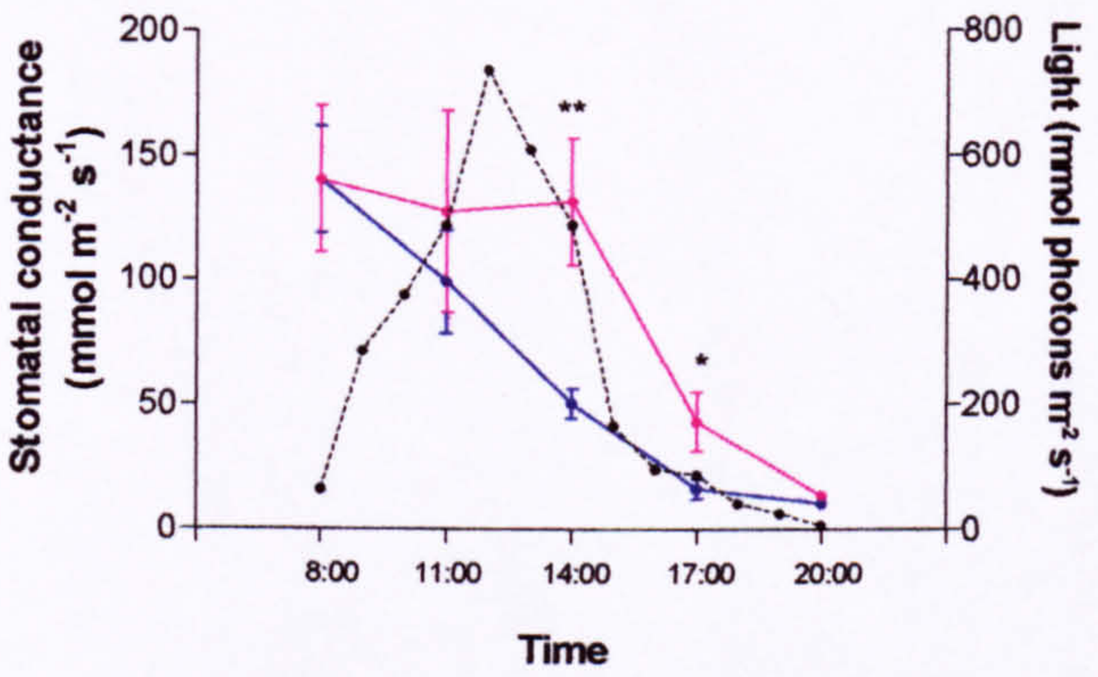


Figure 4.4 Diurnal pattern of stomatal conductance (mean ± SE) after 18 weeks exposure (25th August 01) of *Ligustrum ovalifolium* plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant decrease in stomatal conductance in exhaust gas-polluted air compared with CFA. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted groups (* $p<0.05$; ** $p<0.01$).

Nine weeks into the exposure, there were no significant differences in conductance between treatments in *Hydrangea macrophylla* "Lacecap" plants during the daytime (repeated measure ANOVA, Appendix 31; Figure 4.7). *Hydrangea macrophylla* "Pink" plants in exhaust gas-polluted air had significantly lower conductances compared with clean air controls at midday on day 1 (Figure 4.5), but no overall effect of pollution was detected (repeated measure ANOVA, Appendix 28). During the hours of darkness, however, both varieties of *Hydrangea* exhibited significantly higher conductance in exhaust gas-polluted plants compared with clean air controls (repeated measure ANOVAs, $p=0.007$; Appendix 29 for *Hydrangea macrophylla* "Pink"; $p<0.001$; Appendix 32 for *Hydrangea macrophylla* "Lacecap"). Eighteen weeks into the exposure, in *Hydrangea macrophylla* "Pink" and *Hydrangea macrophylla* "Lacecap", no differences existed between plants in exhaust gas-polluted and filtered air (repeated measure ANOVAs, Appendices 30 and 33; Figures 4.6 and 4.8).

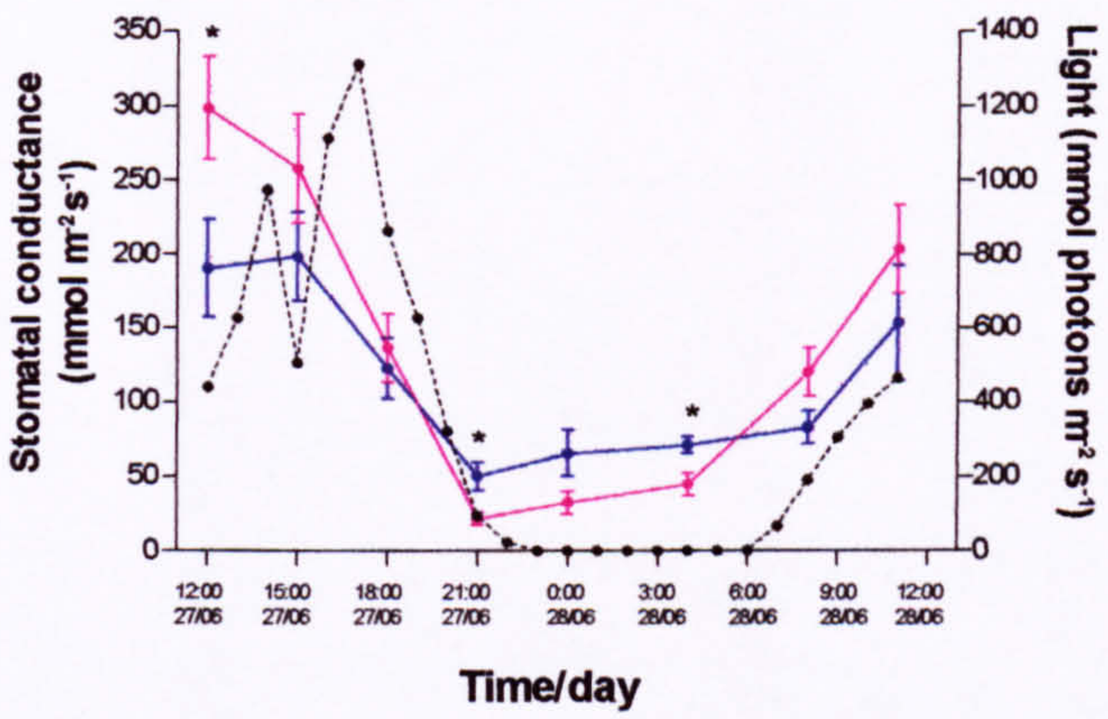


Figure 4.5 Diel pattern of stomatal conductance (mean ± SE) after 9 weeks exposure (27-28th June 01) of *Hydrangea macrophylla* "Pink" plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant increase in stomatal conductance in exhaust gas-polluted air compared with CFA during the night time. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted groups (* $p<0.05$).

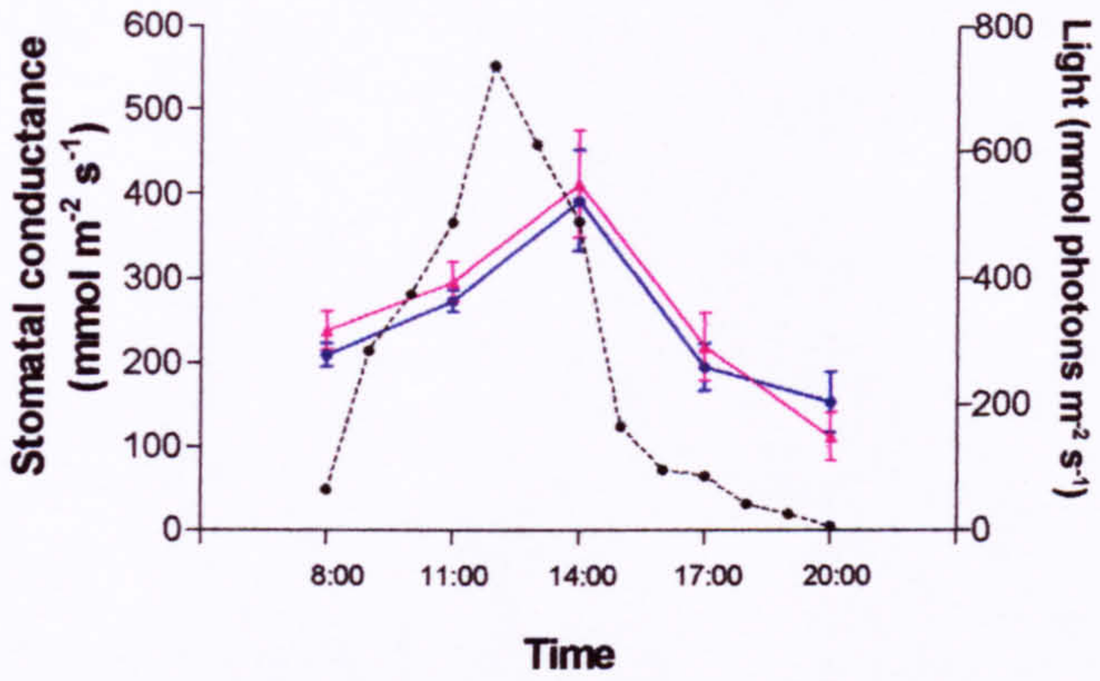


Figure 4.6 Diurnal pattern of stomatal conductance (mean ± SE) after 18 weeks exposure (25th August 01) of *Hydrangea macrophylla* "Pink" plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8.

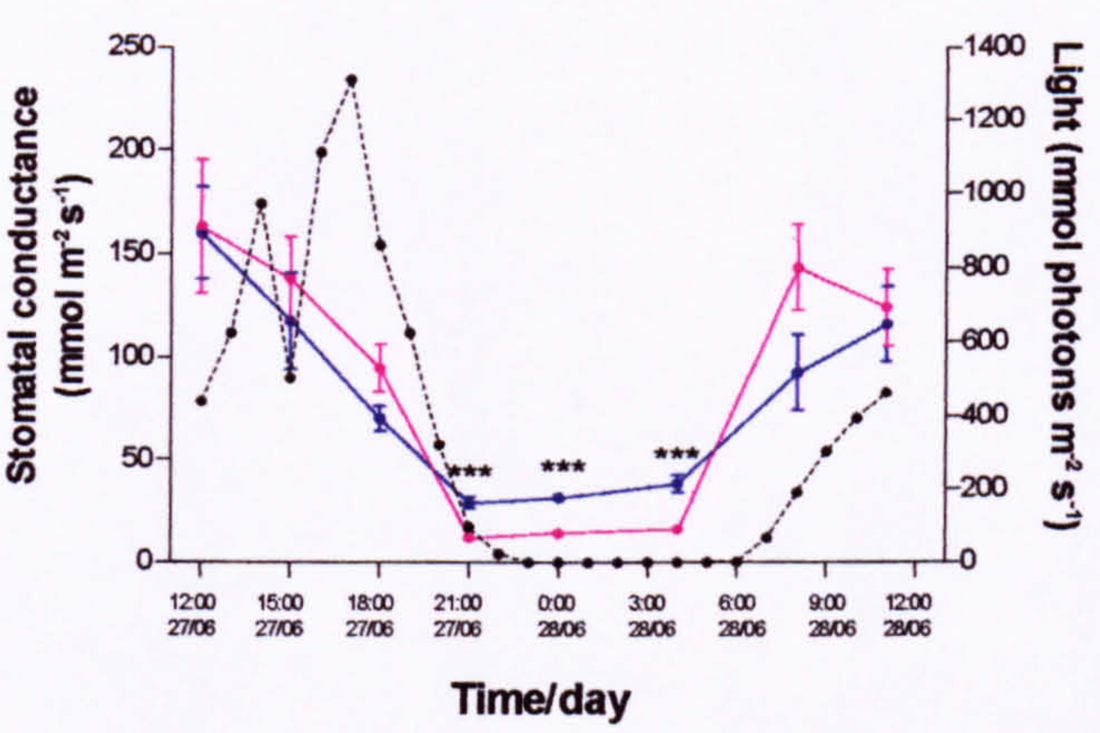


Figure 4.7 Diel pattern of stomatal conductance (mean ± SE) after 9 weeks exposure (27-28th June 01) of *Hydrangea macrophylla* "Lacecap" plants in CFA (●), and in exhaust gas-polluted air (● 100 ppb NO_x). Ambient light (●, dashed line). n=8. A repeated measures ANOVA showed a significant increase in stomatal conductance in exhaust gas-polluted air compared with CFA during the night time. Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted groups (*** $p<0.001$).

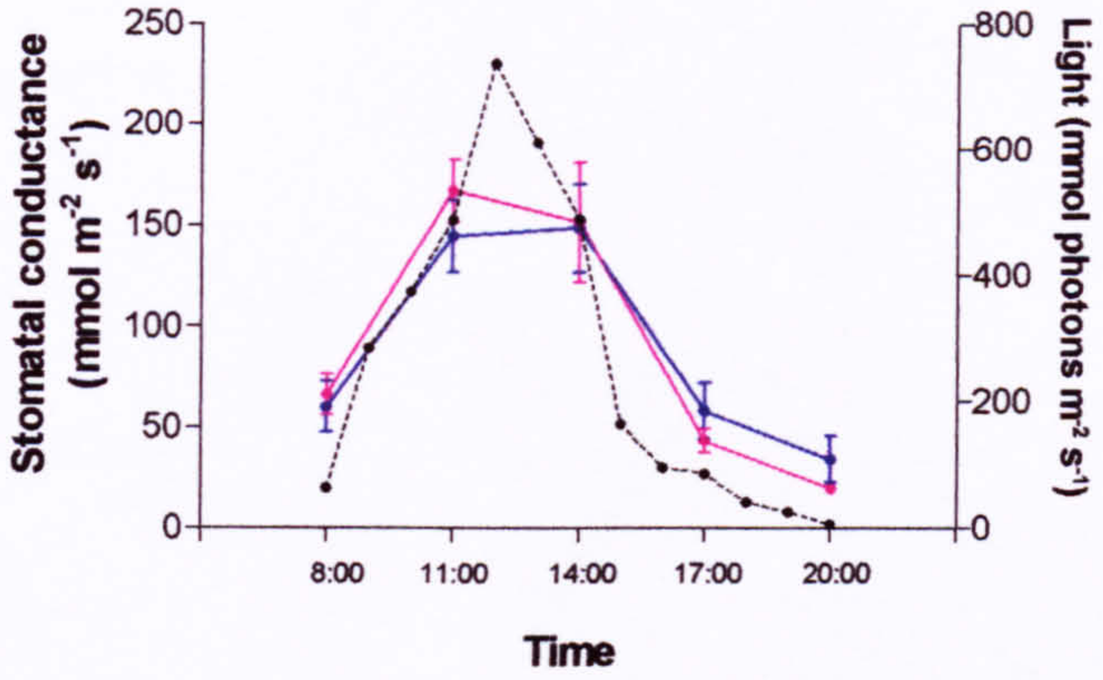


Figure 4.8 Diurnal pattern of stomatal conductance (mean ± SE) after 18 weeks exposure (25th August 01) of *Hydrangea macrophylla* "Lacecap" plants in CFA (•), and in exhaust gas-polluted air (• 100 ppb NO_x). Ambient light (•, dashed line). n=8. Ambient light (σ, dashed line). n=8.

4.3.2 Water status and soil moisture

4.3.2.1 *Cornus sanguinea*

Cornus sanguinea plants subjected to drought wilted 21 days after water was withheld, at a mean water potential of around ¯3 MPa. Exhaust gas-pollution had a significant effect of making water potential less negative (repeated measures ANOVA, $p<0.001$; Appendix 34). From Figure 4.9, this effect of pollution seems to have been more pronounced in non-droughted plants compared with drought-stressed plants. Well-watered plants in polluted air were therefore able to conserve water more effectively than well-watered plants in clean air. Under drought stress, there was no marked difference in water potentials between CFA and exhaust gas-polluted plants. After re-watering, it took the plants four days to return to pre-drought values of water potential. Exhaust gas pollution had no influence on loss of water from the soil (repeated measures ANOVA, Appendix 35; Figure 4.10).

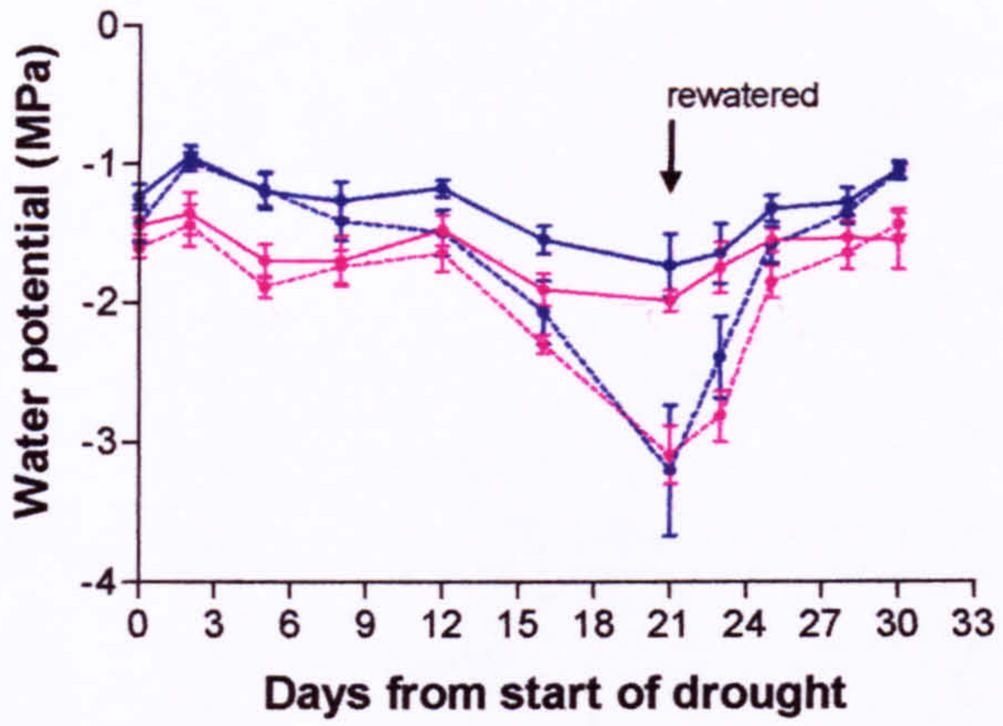


Figure 4.9 Water potential (mean ± SE) of *Cornus sanguinea* plants in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6. A repeated measures ANOVA showed a significant effect of exhaust gas-pollution in making water potential less negative. Within-treatment effects were detected on days 15 for CFA plants and day 21 for CFA and exhaust gas polluted plants.

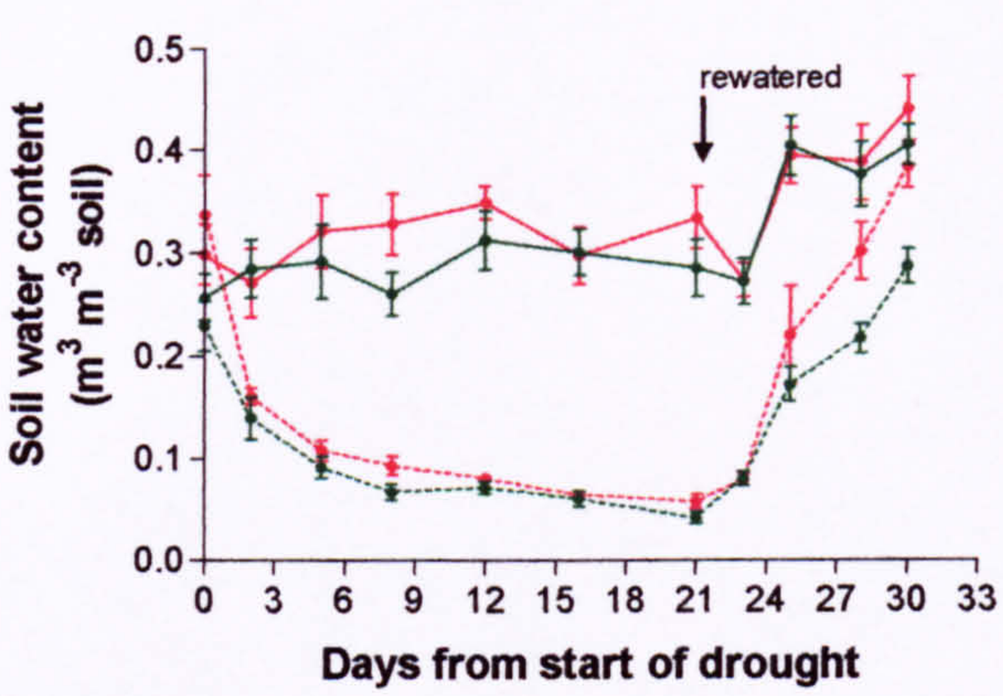


Figure 4.10 Soil moisture (mean ± SE) of *Cornus sanguinea* pots in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6.

4.3.2.2 *Ligustrum ovalifolium*

Exhaust gas pollution had a significant effect on water potential (repeated measures ANOVA, $p<0.001$; Appendix 36) in *Ligustrum ovalifolium*. From Figure 4.11, it is apparent that pollution had the effect of making water potential less negative in both well-watered and drought-stressed plants. The repeated

measures ANOVA (Appendix 36) also shows an interactive effect of pollution and drought ($p=0.015$) on water potential, with pollution decelerating the progression of wilting in drought-stressed plants. The droughted plants in clean air took 15 days to wilt, once they reached a mean water potential of –3.4 MPa. At this point, the droughted plants in polluted air had still not wilted, and had a mean water potential of ¯2.1 MPa. After re-watering, plants recovered extremely rapidly, returning to pre-drought values of water potential within two days. Exhaust gas pollution did not significantly influence the loss of water from the soil during the drought period (repeated measures ANOVA, Appendix 37; Figure 4.12).

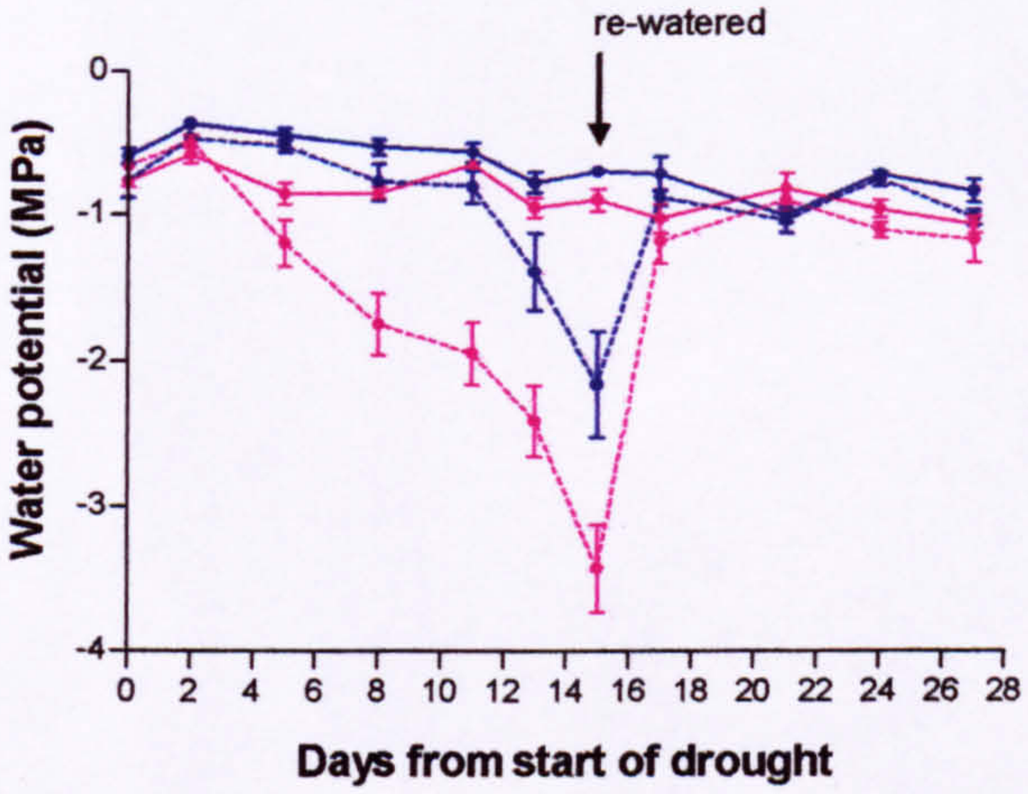


Figure 4.11 Water potential (mean ± SE) of *Ligustrum ovalifolium* plants in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6. A repeated measures ANOVA showed a significant effect of exhaust gas-pollution in making water potential less negative, and an interactive effect of pollution and drought, with pollution lessening the effect of drought on the progression of wilting.

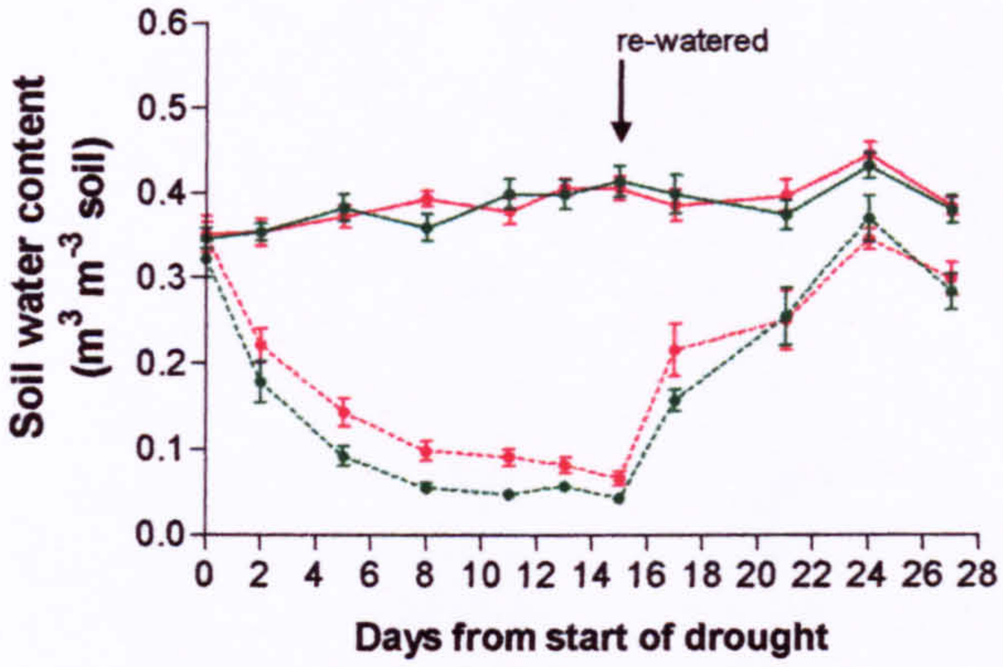


Figure 4.12 Soil moisture (mean ± SE) of *Ligustrum ovalifolium* pots in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6.

4.3.2.3 *Hydrangea macrophylla* "Pink"

In *Hydrangea macrophylla* "Pink", exhaust gas pollution had a significant effect on leaf water potential (repeated measures ANOVA, p=0.029; Appendix 38). From Figure 4.13, it appears that its effect was to generally make water potential less negative, although during the recovery from drought there was a dip in the water potentials of exhaust gas polluted plants that had been subjected to drought. In pots of plants that had been droughted, soil water content did not differ significantly between treatments (repeated measures ANOVA, Appendix 39; Figure 4.14).

4.3.2.4 *Hydrangea macrophylla* "Lacecap"

There were no significant effects of pollution treatment on water status in *Hydrangea macrophylla* "Lacecap" (repeated measures ANOVA, Appendix 40; Figure 4.15). The water-stressed plants wilted after 18 days of drought, at a mean water potential of ⁻1.5 MPa. Plants that had been droughted returned to pre-drought values of water potential within three days of re-watering. Soil moisture levels were also not different between pollution treatments (repeated measures ANOVA, Appendix 41; Figure 4.16).

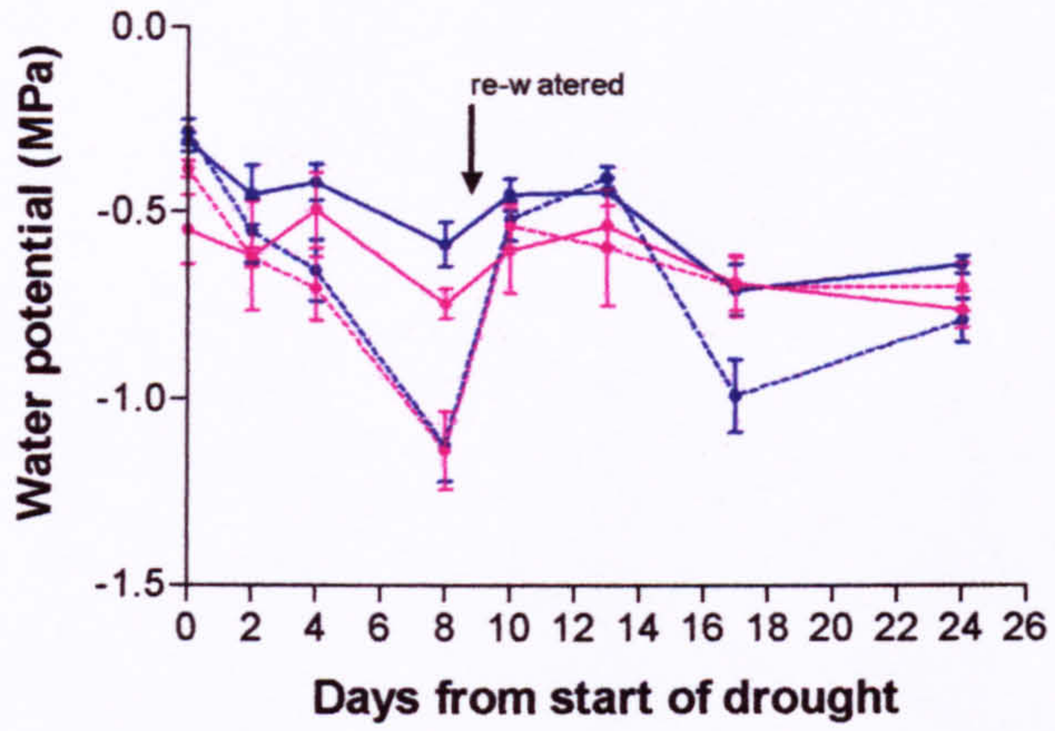


Figure 4.13 Water potential (mean ± SE) of *Hydrangea macrophylla* "Pink" plants in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6. A repeated measures ANOVA showed a significant effect of exhaust gas-pollution in making water potential less negative. Within-treatment effects were detected on days 10 and 13 for CFA plants.

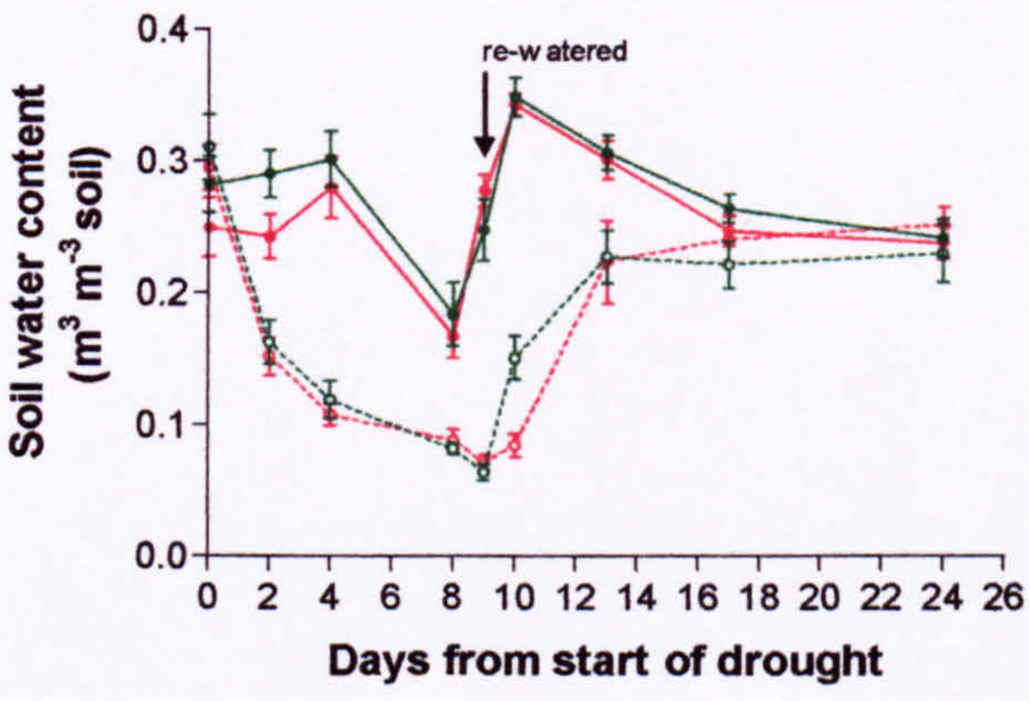


Figure 4.14 Soil moisture (mean ± SE) of *Hydrangea macrophylla* "Pink" pots in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6.

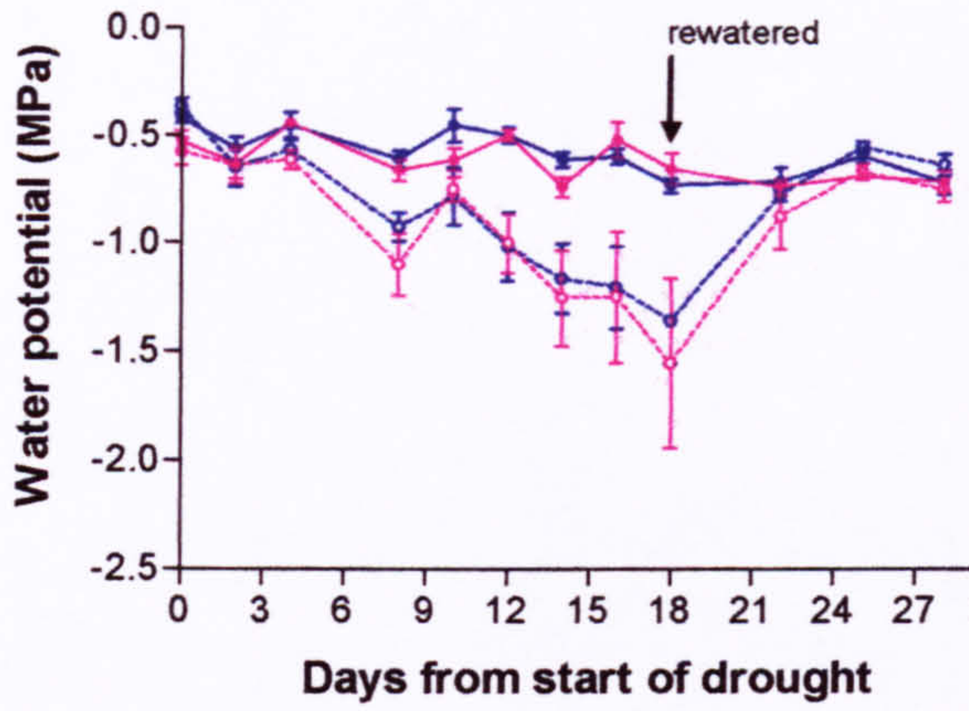


Figure 4.15 Water potential (mean ± SE) of *Hydrangea macrophylla* "Lacecap" plants in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6.

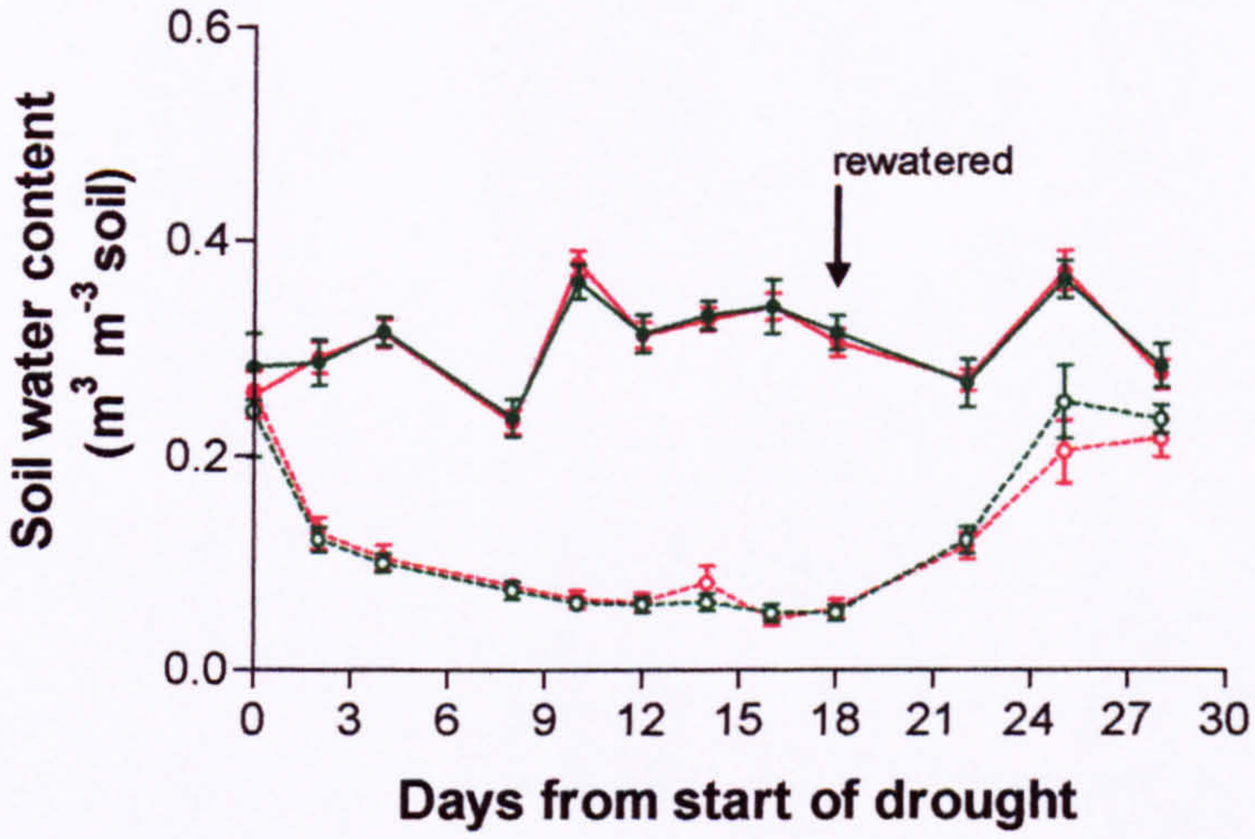


Figure 4.16 Soil moisture (mean ± SE) of *Hydrangea macrophylla* "Lacecap" pots in CFA and exhaust gas-polluted air (100 ppb NO_x). CFA, well watered (●); CFA, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○). n=6.

4.3.3 Stable carbon isotope discrimination ($\Delta^{13}C$)

Values of stable carbon isotope discrimination, $\Delta^{13}C$, for *Ligustrum ovalifolium* leaves following the period of drought are shown in Figure 4.17. There was a significant overall effect of exhaust gas pollution (p=0.021) in decreasing $\Delta^{13}C$

(twoway ANOVA, Appendix 42). There was no significant effect of drought, and no pollution*drought interaction. Using oneway ANOVA and Duncan's multiple range test, the drought treatment had a significant effect on the isotope ratio in plants in CFA, but not in plants exposed to exhaust gas pollution. These results are consistent with the decreased stomatal conductance already observed in *Ligustrum ovalifolium* plants in exhaust gas-polluted air compared with those in clean air (Figures 4.3 and 4.4).

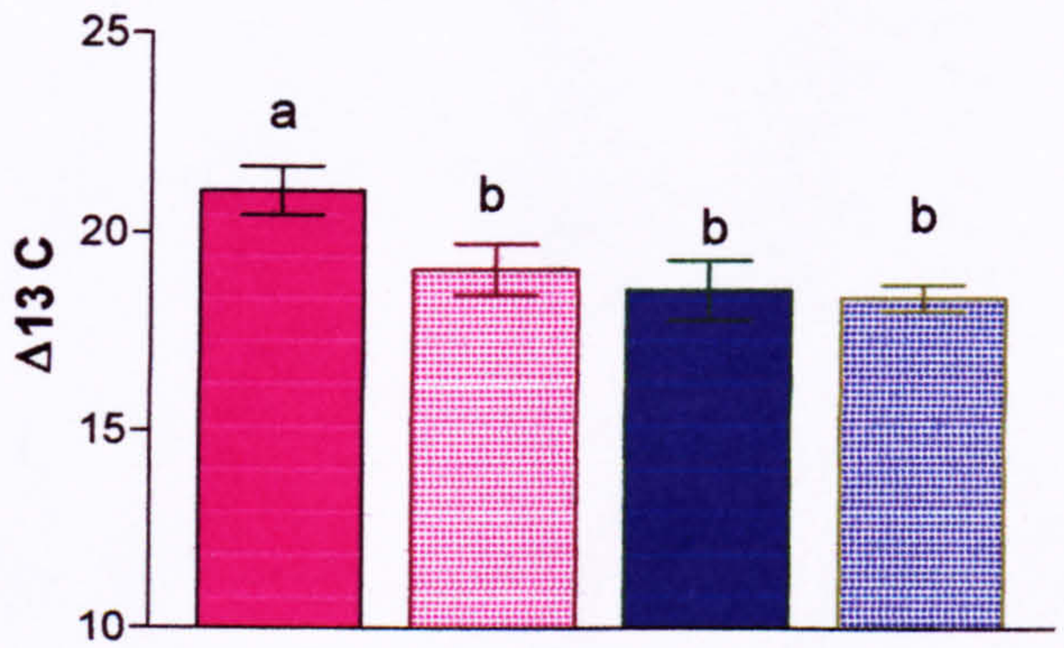


Figure 4.17 Carbon isotope discrimination (mean ± SE) in *Ligustrum ovalifolium* plants plants in CFA and exhaust gas-polluted air (100 ppb NO_x) following an imposed drought. CFA, well watered (■); CFA, not watered (▒); Polluted air, well watered (■); Polluted air, not watered (▒). n=4. Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means.

4.3.4 Growth

Figures 4.18 - 4.21 show the increase in plant height throughout the total exposure period of 14 months. There were no significant differences in these growth parameters between pollution or water treatments, and no pollution*drought interactions were found (repeated measures ANOVAs, Appendices 43-46).

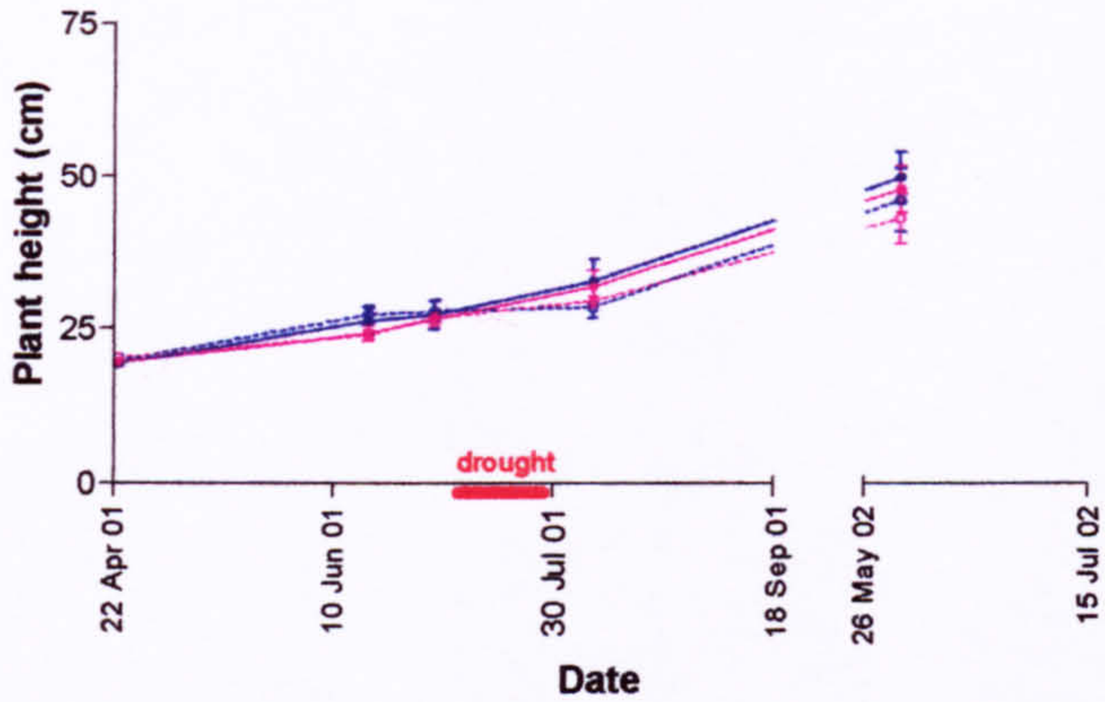


Figure 4.18 Plant height (mean ± SE) in *Cornus sanguinea* plants during exposure to exhaust gas emissions (100 ppb NO_x). Clean air, well watered (●); Clean air, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○); Drought period is indicated by the thickened x-axis (—). n=8.

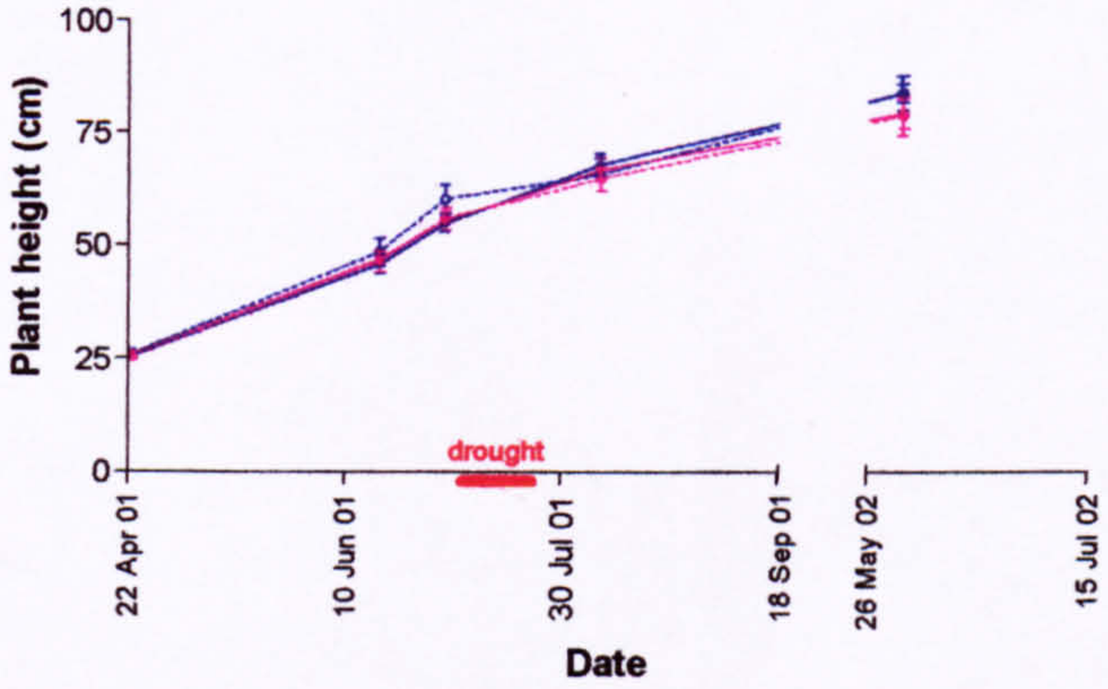


Figure 4.19 Plant height (mean ± SE) in *Ligustrum ovalifolium* plants during exposure to exhaust gas emissions (100 ppb NO_x). Clean air, well watered (●); Clean air, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○); Drought period is indicated by the thickened x-axis (—). n=8.

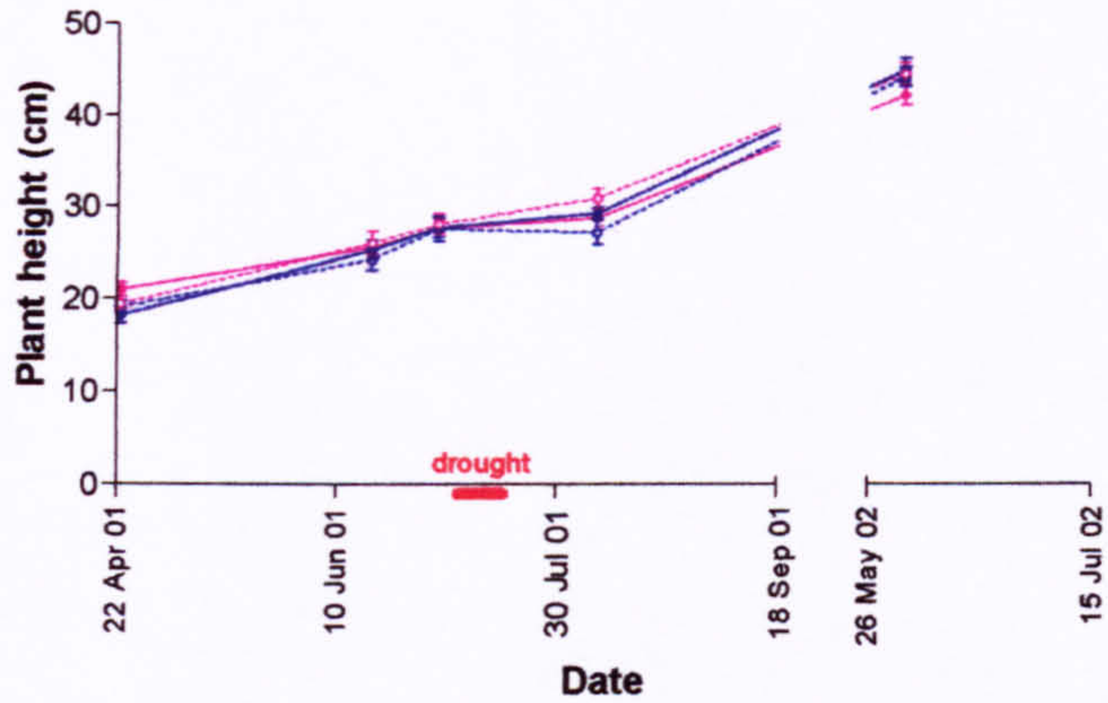


Figure 4.20 Plant height (mean ± SE) in *Hydrangea macrophylla* "Pink" plants during exposure to exhaust gas emissions (100 ppb NO_x). Clean air, well watered (●); Clean air, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○); Drought period is indicated by the thickened x-axis (—). n=8.

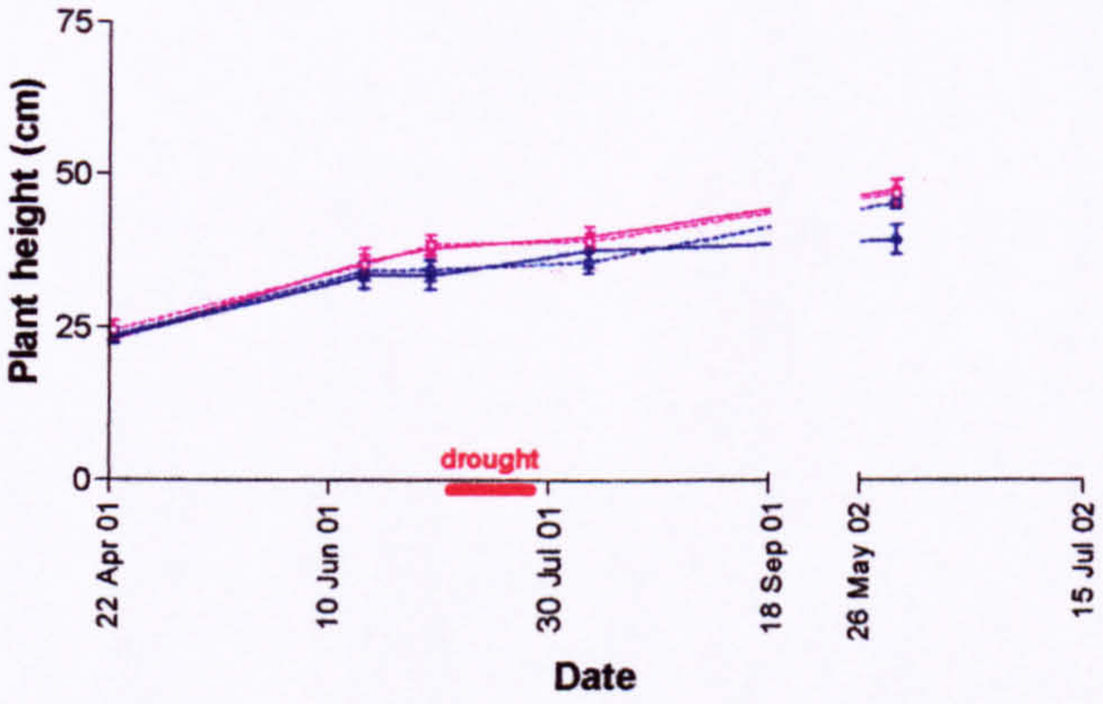


Figure 4.21 Plant height (mean ± SE) in *Hydrangea macrophylla* "Lacecap" plants during exposure to exhaust gas emissions (100 ppb NO_x). Clean air, well watered (●); Clean air, not watered (○); Polluted air, well watered (●); Polluted air, not watered (○); Drought period is indicated by the thickened x-axis (—). n=8.

4.3.5 Biomass

Plant fresh weights and dry weights followed similar patterns between treatments. Therefore, dry weights only are presented here. Dry weights of roots, woody stems, leaves and total above ground biomass are given in Figures 4.22, 4.24, 4.26 and 4.28. Root : shoot ratios are shown in Figures 4.23, 4.25, 4.27 and 4.29.

In both varieties of *Hydrangea*, no differences in biomass existed between any of the treatments (Figures 4.26 - 4.29). Figures 4.23 and 4.24 show the dry weights of *Cornus sanguinea* plants. Summary statistics for dry weights of plant parts of *Cornus sanguinea* are given in Appendix 47. Exhaust gas pollution had a significant stimulatory effect on dry weights of woody stems (twoway ANOVA, $p=0.043$), leaves ($p=0.009$) and total above-ground biomass ($p=0.005$), with the effect of significantly decreasing R:S ($p=0.006$). Drought had no influence on dry weights of plant parts in *Cornus sanguinea*, and there were no pollution*drought interactions.

In *Ligustrum ovalifolium* plants (Appendix 48; Figures 4.24, 4.25), exhaust gas pollution caused a significant decrease in R:S (twoway ANOVA, $p=0.048$). Drought did not have a significant effect on dry weights, and no pollution*drought interactions were apparent.

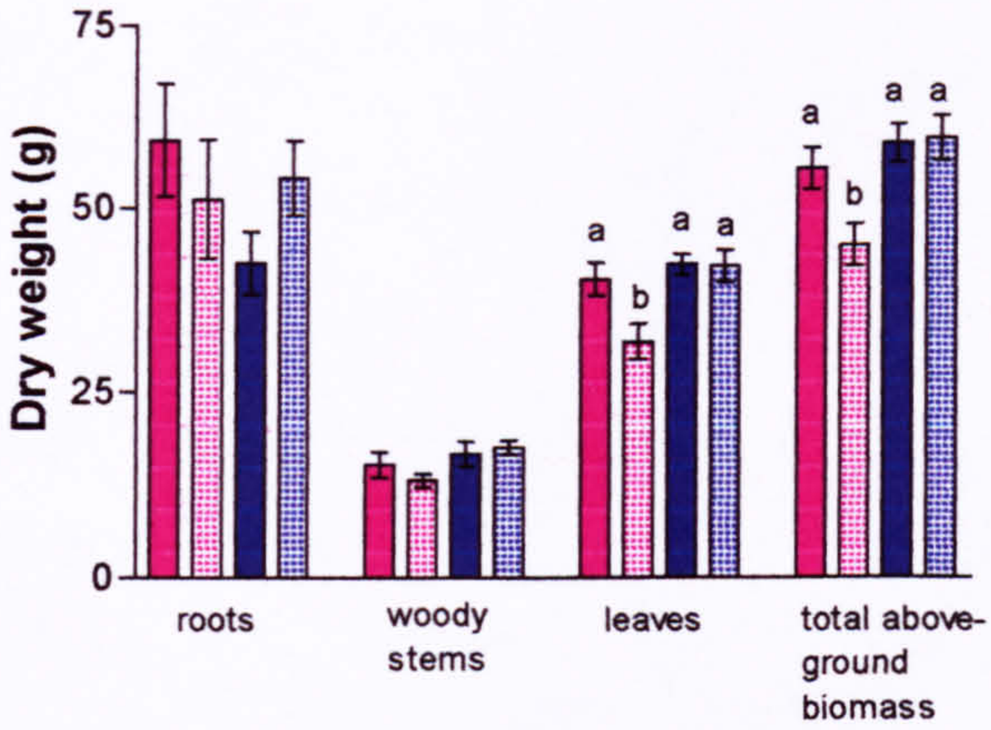


Figure 4.22 Dry weights (mean ± SE) in June 2002 of different parts of *Cornus sanguinea* plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▨); Polluted air, well watered (■); Polluted air, not watered (▨). n=6. Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between mean.

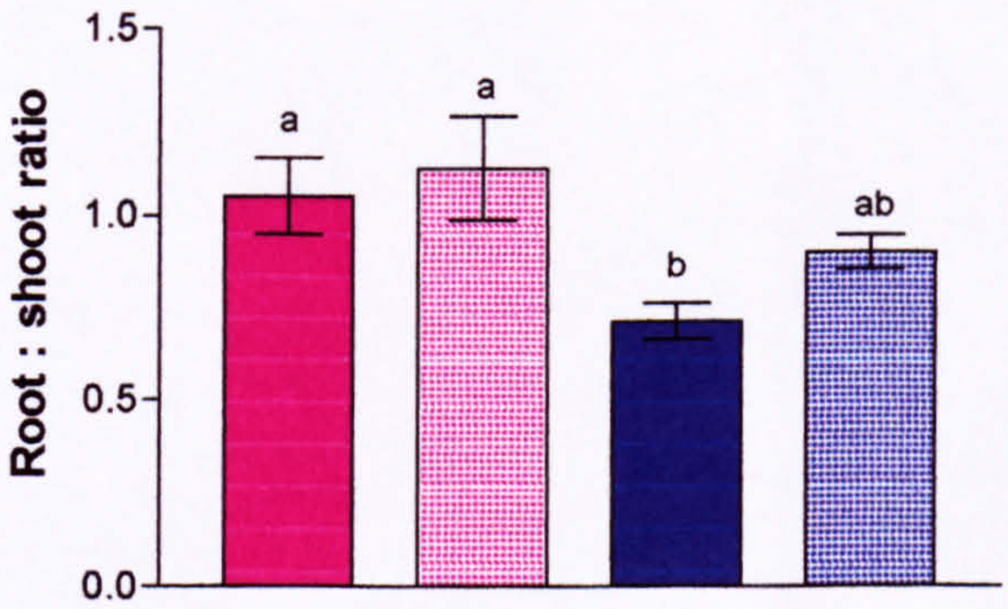


Figure 4.23 Root : shoot ratio (mean ± SE) of *Cornus sanguinea* plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▨); Polluted air, well watered (■); Polluted air, not watered (▨). n=6. Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between mean.

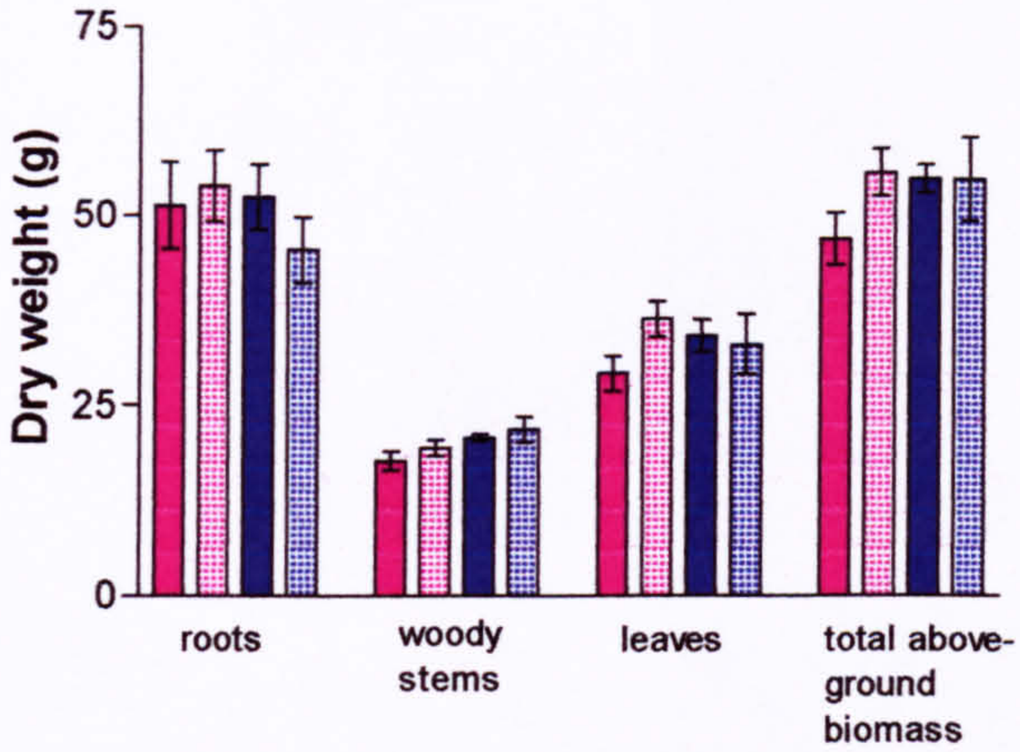


Figure 4.24 Dry weights (mean ± SE) in June 2002 of different parts of *Ligustrum ovalifolium* plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▒); Polluted air, well watered (■); Polluted air, not watered (▒). n=6.

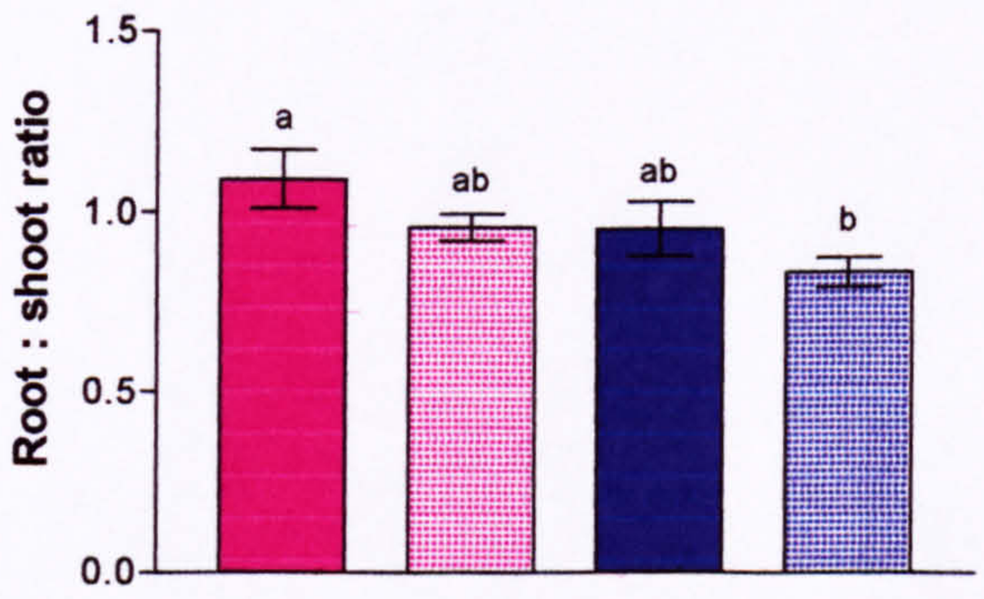


Figure 4.25 Root : shoot ratio (mean ± SE) of *Ligustrum ovalifolium* plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▒); Polluted air, well watered (■); Polluted air, not watered (▒). n=6. Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between mean.

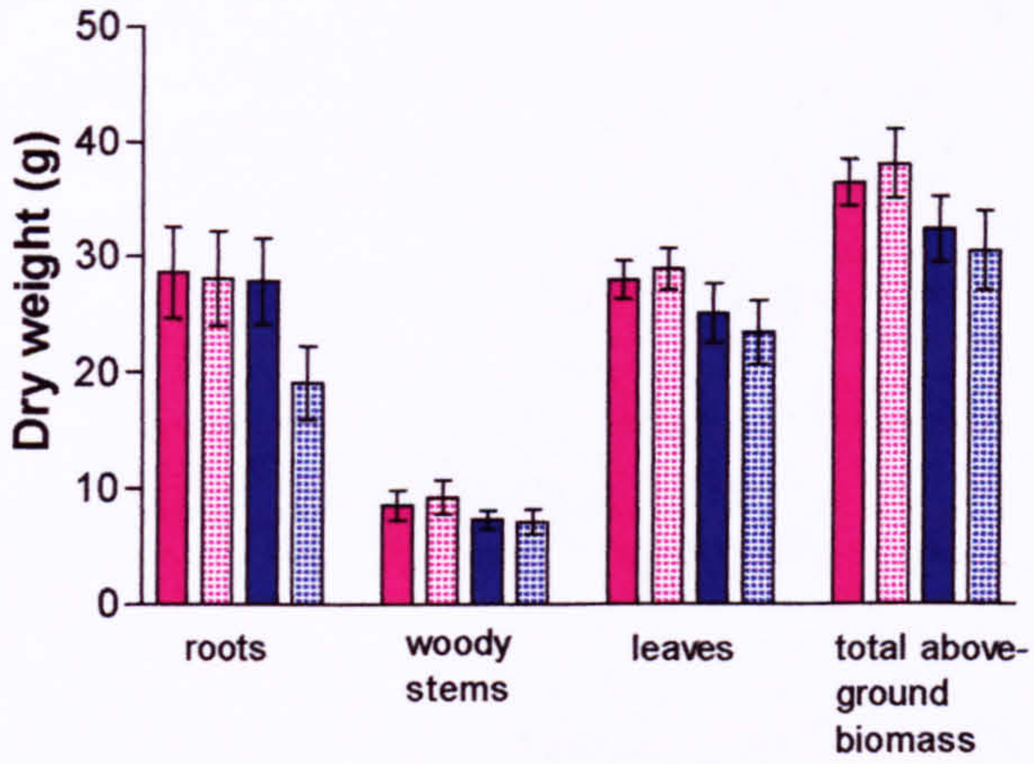


Figure 4.26 Dry weights (mean ± SE) in June 2002 of different parts of *Hydrangea macrophylla* "Pink" plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▒); Polluted air, well watered (■); Polluted air, not watered (▒). n=6.

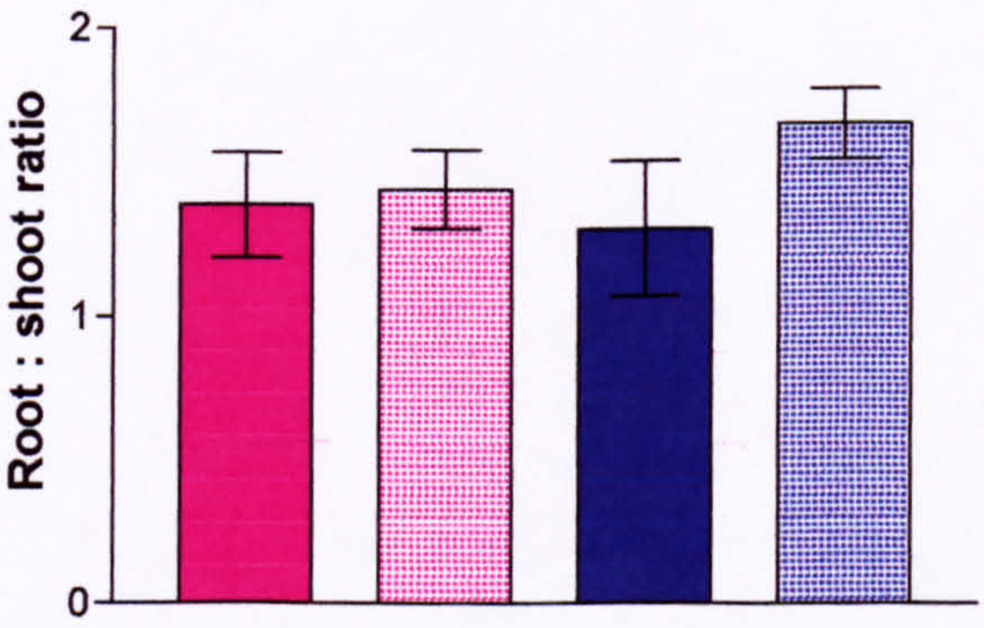


Figure 4.27 Root : shoot ratio ((mean ± SE) of *Hydrangea macrophylla* "Pink" plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered (■); CFA, not watered (▒); Polluted air, well watered (■); Polluted air, not watered (▒). n=6.

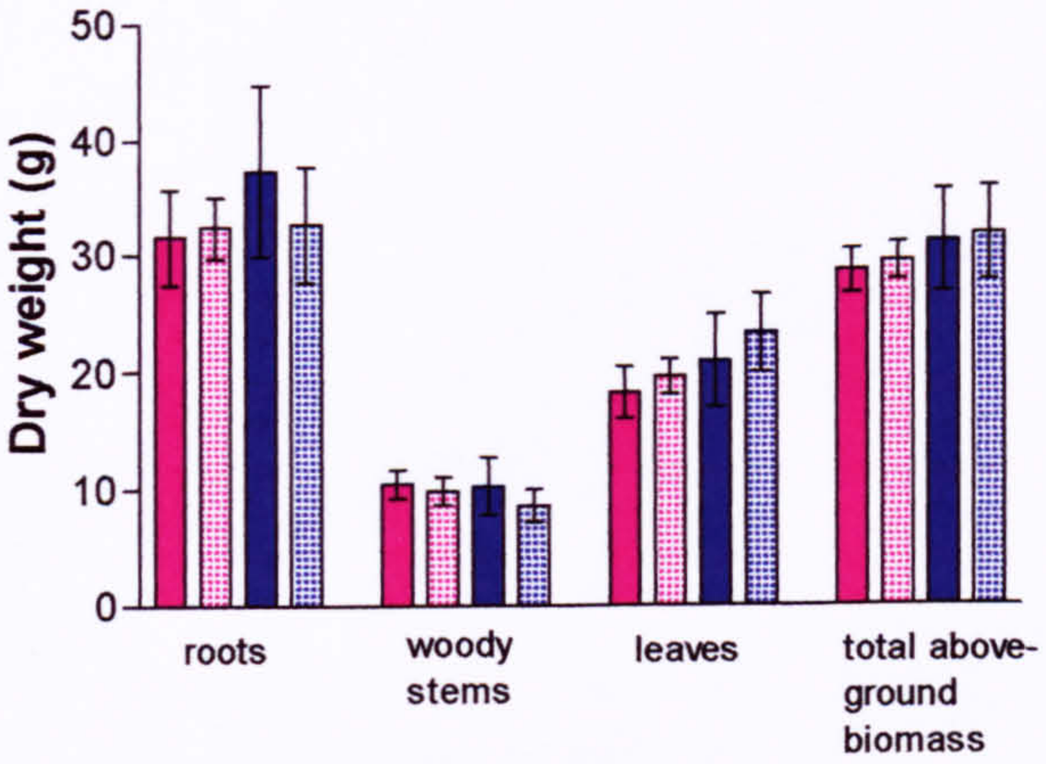


Figure 4.28 Dry weights (mean ± SE) in June 2002 of different parts of *Hydrangea macrophylla* "Lacecap" plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered ( ); CFA, not watered ( ); Polluted air, well watered ( ); Polluted air, not watered ( ). n=6.

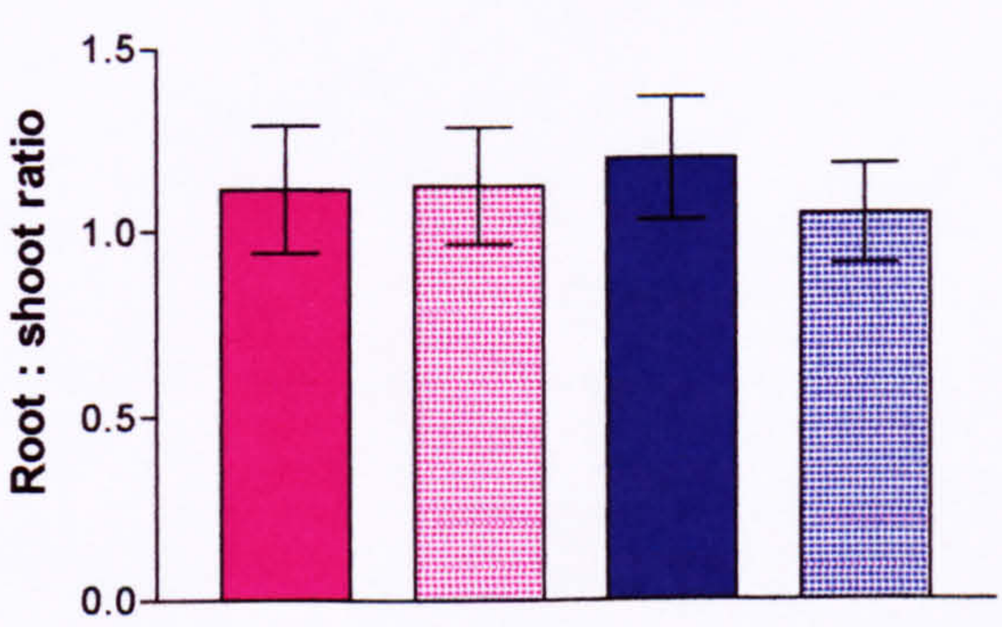


Figure 4.29 Root : shoot ratio (mean ± SE) of *Hydrangea macrophylla* "Lacecap" plants from CFA and exhaust gas-polluted air (100 ppb NO_x) after being subjected to drought during the preceding summer. CFA, well watered ( ); CFA, not watered ( ); Polluted air, well watered ( ); Polluted air, not watered ( ). n=6.

4.4 Discussion

Air pollutants have the potential to alter plant growth and biomass partitioning as well as physiological features such as stomatal behavior and photosynthesis. The direction of alterations in stomatal conductance varies depending on pollution

concentrations and growing conditions, and can influence pollutant flux into the plant and plant responses to other stresses. Effects of pollutants on stomatal conductance and carbon assimilation might be expected cause alterations in growth.

In the present study, urban pollution mixtures did not influence growth, measured by plant height, in any of the species. Fumigation studies with NO_2 and/or NO usually report decreases in plant growth, but in some cases a stimulation of growth has been observed (reviewed in Wellburn, 1990). The UN/ECE and WHO air quality guidelines for total NO_x for all types of vegetation is 30 ppb as an annual mean (NEGTAP, 2000). This value is set to protect the most sensitive components of ecosystems, and so it is possibly not surprising that the species in the present study were able to grow normally at much higher concentrations.

The pollution mixtures affected the allocation of biomass in *Cornus sanguinea* and *Ligustrum ovalifolium*. In *Cornus sanguinea*, the pollution decreased R:S. Air pollutants have been shown to alter biomass partitioning in several studies, and where the effect is a decrease in R:S, this would be expected to have consequences for response to water stress (Mills, 2002). In the present study, the R:S of *Cornus sanguinea* plants was significantly decreased by exhaust gas pollution (through increased allocation to above-ground biomass) but was unaffected by drought. The pollution therefore did not interfere with the plants' drought avoidance capabilities through an influence on R:S in this species.

In *Ligustrum ovalifolium*, exhaust gas pollution again had the overall effect of decreasing R:S, while the drought treatment did not have any significant effect. However, droughted plants in exhaust gas-polluted air had significantly lower R:S compared with well-watered plants in CFA. Such an effect could have long-term implications for drought avoidance, since a lower R:S represents a greater area of transpiring surfaces compared with the surface area available for water

uptake. In the *Hydrangea* varieties, no effects on growth or biomass due to pollution or drought were apparent.

The stomatal response of *Cornus sanguinea* and *Ligustrum ovalifolium* remained constant through the season, with plants in exhaust gas-polluted air having lower rates of conductance compared with clean air controls. Partial stomatal closure is considered to be effective in providing some protection against air pollutants such as NO_x (Lange *et al.*, 1989). A possible cause of stomatal closure under conditions of exhaust gas pollution could be the elevated concentrations of CO_2. Some plants exhibit a strong stomatal closure response to CO_2, which would have the effect of decreasing the uptake of any other pollutants that are present (Robinson *et al.*, 1998). This closure response is often less marked or absent for trees (Robinson *et al.*, 1998; Mansfield, 1998). Elevated levels of CO_2 have been measured in the local atmosphere of cities. In Edinburgh city centre, CO_2 concentrations ranged from 354 ppm to 416 ppm, compared with global atmospheric background concentrations of 360 ppm. Concentrations showed a diurnal cycle, with peaks at rush-hours (Nemitz *et al.*, 2002). Day *et al.* (2002) conducted a study of CO_2 concentration in Phoenix, Arizona, a particularly polluted city. CO_2 concentrations in the urban center were 8 ppm higher than at the edge of the metropolitan area during the daytime (396 ppm compared with 377 ppm), and 24 ppm higher at night (409 ppm compared with 385 ppm). These studies demonstrate that CO_2 levels in urban atmospheres could be sufficient to cause a stomatal response in some plants.

Stomatal conductance peaks at different times of the day in different plant species. It has been suggested (e.g. Musselman and Minnick, 2000) that the timing of these peaks could be significant in terms of the effective dose of O_3 entering the plant, because there are also diurnal variations in O_3 concentrations. The same principle could also apply to primary traffic pollutants, as already discussed for CO_2. In urban centres on weekdays, NO_x concentrations peak between 08:00 and 09:00 and again in the late afternoon (i.e. at rush hours). At

weekends the pattern is different, with lower overall NO_x concentrations and a daytime peak occurring between 11:00 and 12:00. There are also increases in nighttime traffic flow at weekends and a nighttime peak in NO_x (e.g. Almbauer *et al.*, 2000; Morawska *et al.*, 2002). When conditions favor photochemical reactions involving NO_x, the diurnal concentration pattern is altered (e.g. Isidrov Aleroso *et al.*, 1992). On such days, NO_x are consumed rapidly following their morning peak, contributing to the production of O_3.

Cornus sanguinea plants reached high rates of stomatal conductance early in the day, which in an urban situation would correspond to the morning rush hour and high concentrations of exhaust gas pollutants. However the urban pollution mixtures had the effect of decelerating stomatal opening in the morning, reducing the peak in stomatal conductance in the late morning, and speeding up stomatal closure in the late afternoon/early evening. A similar though less marked pattern occurred in *Ligustrum ovalifolium*. The effect of the pollutants in decreasing conductance in these species (possibly mediated by CO_2) would therefore have the side effect of reducing the dose of pollutant gases at the times of day when concentrations would be at their highest.

The *Hydrangea* varieties did not show an obvious stomatal response to exhaust gas pollution during the day, but by night conductance was higher in plants growing in exhaust gas-polluted air compared with those in clean air. This nocturnal increase in stomatal opening in response to air pollutants agrees with the findings of Viskari *et al.* (2000b) where spruce seedlings were fumigated with vehicle exhaust gases. Numerous studies have documented stomatal activity during the night (reviewed in Musselman and Minnick, 2000), but the causes and the consequences of this are not fully understood.

The greater stomatal conductance in response to exhaust gas pollution observed in the present study could reflect a failure of stomata to close completely. However, since *Hydrangea macrophylla* "Pink" plants in exhaust gas-polluted

air had significantly lower conductances at midday compared with clean air controls, it seems unlikely that their stomatal closure mechanism had been impaired by the pollution. It is possible that the elevated nocturnal conductance reflects enhanced respiration rates, which might indicate an effort by the plant to repair pollution-induced injury. However, respiration was not measured in the present study. Plants with open stomata would be exposed to a higher dose of pollutants. In the *Hydrangea* varieties, pollution-induced stomatal opening occurred during the nighttime. At night, pollution concentrations in urban atmospheres are generally low, except possibly at weekends when NO_x concentrations can have a nocturnal peak, so that nighttime stomatal opening might not be important in terms of pollution flux.

The two species showing the most obvious stomatal responses to urban pollution mixtures (i.e. *Cornus sanguinea* and *Ligustrum ovalifolium*) also had more marked responses to drought stress. In *Cornus sanguinea*, leaf water potentials were less negative in polluted air compared with CFA when the plants were not under water stress. This could be the result of the partial stomatal closure already observed in the plants in polluted air, which would have the effect of decreasing transpirational water loss. However, in the non-watered plants, as the water potentials began to drop in response to the drought, any difference between exhaust gas-polluted plants and CF plants disappeared, so that the pollution did not appear to have any influence on drought avoidance capabilities in this species.

In *Ligustrum ovalifolium*, plants in exhaust gas-polluted air had less negative water potentials compared with those in CFA under both drought and non-drought conditions. The droughted plants showed a great difference in water potentials between CFA and polluted air, with a difference of around 1 MPa. When the plants in CFA reached the point of wilting, the plants in exhaust gas-polluted air still had relatively turgid leaves. The pollution therefore seemed to afford the plants some protection against drought stress (at least in the short-

term), probably through its influence on stomatal conductance and therefore water loss. Analysis of carbon isotope discrimination, an integrated measure of stomatal conductance, showed that the pollution did have a prolonged effect in decreasing gas exchange in this species.

In the *Hydrangea* varieties, there were no marked differences in water status in response to the pollution under either well-watered or drought conditions. In *Hydrangea macrophylla* "Pink", eight days after the droughted plants had wilted and had been re-watered, plants in polluted air that had been droughted showed a dip in water potentials compared with droughted plants in CFA. It is possible that this represents a lag in recovery from drought stress in the plants in polluted air.

This study has identified two species of shrub, *Cornus sanguinea* and *Ligustrum ovalifolium*, that exhibit responses to urban pollution mixtures. Water status in *Ligustrum ovalifolium* exhibited an interactive response to pollution in combination with water stress. In the short-term, exhaust gas pollution afforded protection to *Ligustrum ovalifolium* plants against loss of turgor and caused a delay in wilting, probably through its effect on stomatal conductance. In the longer term however, the pollution caused shifts in biomass partitioning in plants that had been subjected to drought. The droughted plants in polluted air had significantly lower R:S compared with well-watered plants in CFA. This decrease in R:S would be expected to impair long-term drought avoidance capabilities.

Chapter 5: The Influence of Nitrogen Deposition on Plant Response to Urban Pollution Mixtures

5.1 Introduction

5.1.1 Effects of enhanced nitrogen deposition on ecosystems

Natural and semi-natural ecosystems in or close to urban centres are subjected to nitrogen enrichment from the application of fertilizers to planted vegetation, and from the deposition of NO_x and NH_x from vehicular emissions. Nitrogenous pollutants are delivered to ecosystems via dry and wet deposition and through the deposition of fog and cloud water (e.g. Lovett, 1994). No figures are available of rates of nitrogen deposition specifically to urban and surrounding areas. In Europe, the combined atmospheric nitrogen deposition from ammonia (from intensive agricultural systems) and from NO_x to non-forest ecosystems can be 20 – 60 Kg N ha^{-1} y^{-1}. (Figure 1.1 shows total emission of nitrogen in the UK). In the early 1900s, it is estimated that background inputs were only 1 – 3 Kg N ha^{-1} y^{-1} (Bobbink and Lamers, 2002). Forest edges close to agricultural or urban areas have been found to scavenge nutrients and pollutants, with up to four-fold increases in nitrogen deposition rates compared with forest interiors or open grassland (e.g. Weathers *et al*, 2001). Therefore trees and shrubs in or near the city might be expected show similarly enhanced deposition rates to those of forest edges.

Increased input of nitrogen into an ecosystem can lead to complex changes in its structure and functioning (Bobbink and Lamers, 2002). In nitrogen-poor habitats, where nitrogen is a limiting nutrient for plant growth, its addition can lead to changes in competitive ability and plant species composition (e.g. Tamm, 1991; Aerts and Chapin, 2000). Such habitats include most forests in temperate climates, where nitrogen deposition has been identified as a possible factor in promoting forest growth (e.g. Davison and Barnes, 2002). Also in the heathlands of upland Britain, it is considered that eutrophication may be causing heather, mosses and lichens to lose their competitive advantage over other plants such as grasses and bracken (e.g. Rose, 1994; Gordon *et al.*, 1999).

Ecosystems in Europe have been classified according to sensitivity to nitrogen deposition, based on vegetation types (e.g. Kuylenstiema *et al.*, 1998, using examples of critical loads from Grennfelt and Thörnelöf, 1992 and Hornung, 1995). Under this classification, birch, beech and various species of oak were in a low sensitivity class, able to withstand deposition rates of 15-30 Kg N ha^{-1} y^{-1}. More recently, these values have been revised to give critical loads affecting the most sensitive components of ecosystems. In the case of temperate forest habitats, perhaps the best guide for urban trees/woodland, this is the ground flora, which is affected by deposition rates of 10-15 Kg N ha^{-1} y^{-1} (Bobbink *et al.*, 2002).

5.1.2 Effects of enhanced nitrogen deposition on plants

The physiological basis of responses of plants to increased nutrient supply is not fully understood (Padgett and Allen, 1999). Physiological responses vary between plant species and functional types. In 1-year old *Cryptomeria japonica* seedlings subjected to gradations from 0 – 340 Kg N ha^{-1} y^{-1}, net photosynthetic rate increased with the level of nitrogen addition (Nakji *et al.*, 2001). Net photosynthesis of *Pinus densiflora* seedlings under the same nitrogen regimes was significantly reduced in the highest nitrogen treatment (Nakji *et al.*, 2001). In *Calluna vulgaris* and *Pteridium aquilinum*, photosynthesis, transpiration and water use efficiency were not affected by the addition of up to 50 Kg N ha^{-1} y^{-1} (Whitehead *et al.*, 1997).

At the whole-plant level, enhanced nitrogen availability tends to decrease the root : shoot ratio (R:S), a result of decreased root growth and/or increased resource allocation to the shoot (Matzner and Murach, 1995). At high nitrogen availability, the plant is effectively carbon-limited, and so maximizes carbon allocation through increased investment in the shoot (e.g. Hättenschwiler and Körner, 1997). Nitrogen-induced shifts in resource allocation could negatively impact plants, since other nutrients may become relatively limited, leading to secondary deficiencies (e.g. Miller and Miller, 1988). Resistance to stresses such

as drought can be impaired, for example in heather where nitrogen-stimulated shoot growth increased water demand to the point where the plants were less able to withstand conditions of low water supply (Gordon *et al.*, 1999).

Air pollutants such as O_3 have been shown to interfere with normal senescence processes in plants, characterized by for example chlorophyll breakdown, declines in net RUBISCO and ultimately leaf abscission (e.g. Bielenberg *et al.*, 2001; Mikkelsen and HeideJorgensen, 1996). In the case of poplar, this O_3 effect was exacerbated by decreasing the soil nitrogen supply (Bielenberg *et al.*, 2001). Exposure of *Lotus corniculatus* to a mixture of VOCs has been found to cause premature senescence by advancing the timing of seed pod production (Cape *et al.*, 2003b). Norway spruce exposed to exhaust gas emissions showed signs of premature senescence related to alterations in mesophyll ultrastructure (Viskari *et al.*, 2000b). It is therefore possible that exposure to urban pollution mixtures and level of nitrogen deposition could interact to alter the rate of senescence, as has been found for O_3 (Bielenberg *et al.*, 2001). Early senescence is indicative of a shortened period of carbon acquisition, and is therefore expected to have a negative impact on the plant (Bielenberg *et al.*, 2001). In the case of perennial deciduous species, this could have implications for growth in the following year.

Some trees, e.g. spruce and beech, have been found to decrease their uptake rates of NO_3^- and NH_4^+ when subjected to high loads of nitrogen, partially counteracting the effects of nitrogen deposition (Näsholm, 1998). However, whereas root nitrogen uptake can be down-regulated depending on the availability of nitrogen, shoot uptake of gaseous nitrogen compounds cannot be shut down in a similar way (Näsholm, 1998). There does seem to be a signaling mechanism between the shoot and root (Rowland *et al.*, 1985), so that enhanced NO_2 uptake by the shoot significantly decreases root nitrogen acquisition (Muller *et al.*, 1996).

Nitrate reductase (NR) and nitrite reductase (NiR) are the first enzymes involved in the assimilation of nitrogen, and can therefore reflect rates of nitrogen uptake by the plant. In some plants, the presence of NO_x decreases the activity of NR while increasing that of NiR. This is thought to be due to the dissolution of NO_2 and NO in the extracellular water. Particularly with NO, this provides a supply of nitrite ions so that there is less need for their production by NR (Capron and Mansfield, 1976; Mansfield and Lucas, 1996). More NiR is required in order to remove the nitrite ions that are present (Mansfield and Lucas, 1996). In contrast, the presence of NO_2 in the air has been shown to increase NR activity in several plant species (reviewed in von Ballmoos *et al.*, 1998). If more NO_2 compared with NO enters into solution in the cell, this gives rise to more nitrate ions in comparison with nitrite, with the effect of inducing NR activity. Induced increases in NR activity are a result of de-novo synthesis of the enzyme (von Ballmoos *et al.*, 1998). The ability to induce NR in response to NO_2 might provide a degree of resistance to NO_x pollution, since excess levels of nitrite ions are toxic to plant tissues (Capron and Mansfield, 1976).

von Ballmoos *et al.* (1998) looked at the effects on nitrogen uptake of gaseous NO_2 and soil nitrogen fertilization in 4-year old Norway spruce trees. Fumigation with 100 $\mu g\ m^{-3}$ NO_2 led to a significant long-term increase in NR activity in the needles. Fertilizing the trees with 5mM NPK nutrient solution did not affect the NR activity in the needles. *Phaseolus vulgaris* showed a different response, having an increase in shoot NR activity with increasing root nitrate supply. NR activity was increased by the presence of atmospheric NO_2, but only when soil nitrate availability was low (Srivastava and Ormrod, 1984). Rowland *et al.* (1987) looked at the effect of atmospheric NO_2 and nitrate availability in the nutrient solution on NR activity in both the shoots and roots of hydroponically-grown barley. Root NR activity did not react to the presence of NO_2 but, at low nitrate availability, shoot NR activity increased with higher concentrations of NO_2.

5.1.3 Aims of this study

This study aimed to test the responses of two species of commonly-planted shrub to exhaust gas pollution mixtures and different levels of simulated nitrogen deposition. This was intended to mimic the simultaneous exposure to gaseous pollutants, and wet and dry deposition of nitrogenous pollutants experienced by vegetation in urban areas. It also allowed a separation of the effects of gaseous pollutants and deposited forms of nitrogen.

5.2 Materials and methods

5.2.1 Plant material

Sixty four plants of each of two species (*Cornus sanguinea* and *Ligustrum ovalifolium)* were purchased from from Cheviot Trees (Berwick upon Tweed, Northumberland), potted as described in Section 2.2.1, and placed in the Solardomes on 27 May 2002. Sixteen plants were assigned to each dome, and these were equally divided into four simulated nitrogen deposition treatments. Plants were pruned to standard size at the start of the experiment.

5.2.2 Addition of nitrogen

Nitrogen deposition was simulated by the addition of NH_4NO_3 dissolved in distilled water. The 16 plants of each species in each Solardome were divided into four groups, each containing four plants. The groups received solutions of NH_4NO_3 that gave the equivalent of 0, 10, 20 and 50 Kg N ha^{-1} y^{-1}. Nutrient solutions were added at 14-day intervals from 6th June 2002 – 12th September 2002.

5.2.3 Stomatal conductance

Measurements of stomatal conductance were taken on 17th abd 18th June 2002, according to the method given in Section 2.2.2.

5.2.4 Gas exchange and Photosynthesis

Gas exchange and photosynthesis measurements were made on the second-eldest leaf using the method described in Section 2.2.3. A set of measurements were taken between 16th-19th July and another between 13th-19th August 2002.

5.2.5 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were made on the second eldest leaf on 13th-19th August as described in Section 2.2.4.

5.2.6 Nitrate reductase (NR) activity

On 26th-29th September 2002, leaf or root samples for determination of NR activity were collected from plants in the Solardomes between 08:30 and 09:30, placed in sealed plastic bags and transported immediately to the laboratory. NR activity was determined as described in Section 2.2.9.

5.2.7 Growth

Plant height was measured throughout the experiment.

5.2.8 Biomass

In October 2002, the plants were harvested, separated into roots, woody stems and leaf material, and weighed. They were then dried to constant weight at 80 °C and re-weighed. *Cornus sanguinea* plants had lost their leaves, and so woody stems and roots only were harvested.

5.2.9 Analysis of foliar nitrogen concentration

Leaves collected for nitrogen analysis on 25th October were oven-dried at 80°C and ground in a mill. Samples (5-10 mg) were analysed using a RoboPrep Biological Sample Converter (Europa Scientific Inc., London, UK) by E. J. Okello of the Analytical Services Consultancy, School of Biology, University of Newcastle.

5.2.10 Leaf retention near the end of the growing season

The numbers of leaves remaining on the plants were counted on 24th September 2002.

5.2.11 Senescence in *Cornus sanguinea* determined by leaf colour

Using plants from the zero additional nitrogen treatment (0 Kg N ha^{-1} y^{-1}), the numbers of leaves exhibiting different percentage leaf areas covered by red pigment were counted in each pollution treatment on 24th September 2002.

5.2.12 Senescence in *Cornus sanguinea* determined by chlorophyll fluorescence

Plants from the zero additional nitrogen treatment (0 Kg N ha^{-1} y^{-1}) were used for fluorescence measurements (Section 2.2.4) on leaves of different age classes on 25th September 2002.

5.2.13 Statistical analysis

Statistics were performed using a standard SPSS statistics package (SPSS Inc., Chicago, USA). Data were checked for normal distribution and homogeneity of variance, then tested for chamber effects within treatments using ANOVA with Duncan's multiple range test. Few chamber effects were found, and are indicated in graph legends. Data from the different replicate chambers were pooled together. Using the pooled data, twoway ANOVA were used to detect differences between pollution and nitrogen treatments, and to test for pollution*nitrogen treatment interactions. Any differences were further investigated using oneway ANOVA with Duncan's multiple range test. For measurements taken over a time course, repeated measures ANOVAs were employed, factors being time and treatment(s).

For numbers of leaves exhibiting different percentage cover of red pigmentation, the data from the replicate chambers were pooled and compared using the χ^2 statistic.

5.3 Results

5.3.1 Stomatal conductance

Figure 5.1 shows stomatal conductance values at ambient conditions in *Cornus sanguinea* plants over the course of a day in CFA and exhaust gas-polluted air under different nitrogen addition regimes. Neither exhaust gas pollution nor nitrogen deposition had any significant effects on stomatal conductance (repeated measures ANOVA, Appendix 49; Figure 5.1).

In *Ligustrum ovalifolium* (Figure 5.2), neither exhaust gas pollution nor nitrogen deposition had any significant overall effects on stomatal conductance (repeated measures ANOVA, Appendix 50, Figure 5.2). However at 13:00, plants in the 0 Kg N ha^{-1} y^{-1} treatment exhibited significantly lower conductance in exhaust gas-polluted air compared with their counterparts in CFA (oneway ANOVA, $p<0.05$).

5.3.2 Gas exchange and photosynthesis

Assimilation (A_{sat}) and stomatal conductance (Gs_{sat}) at saturated light (PPFD = 800 μmol m^{-2} s^{-1}) were measured on 16-19th July and 13-19th August 2002. In July, no effects of pollution or nitrogen treatment were detected in either species (Figures 5.3 – 5.6). By August, assimilation and stomatal conductance rates were considerably lower in both species compared with rates measured in July, probably due to a deceleration in growth (Figures 5.7, 5.8, 5.9 and 5.10). In *Cornus sanguinea*, A_{sat} underwent a more marked reduction from July to August in exhaust gas-polluted plants in the two lowest nitrogen treatments (0 and 10 Kg N ha^{-1} y^{-1}) compared with CFA/higher nitrogen treatments (Figure 5.7). In *Ligustrum ovalifolium* plants, the situation was similar in July and August, with no differences in A_{sat} or Gs_{sat} between treatments (Figures 5.9 and 5.10).

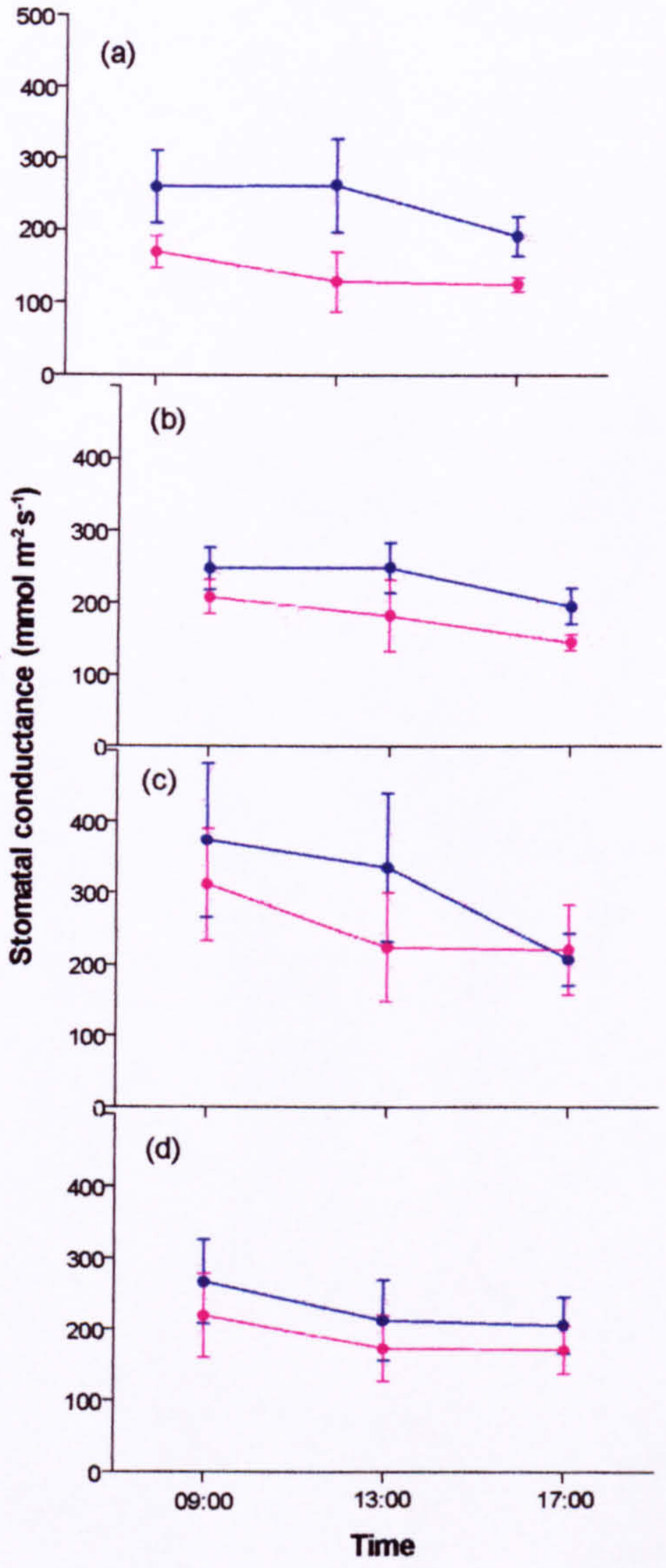


Figure 5.1 Stomatal conductance (mean ± SE) in *Cornus sanguinea* plants in CFA (●) and exhaust gas-polluted air (●; 100 ppb NO_x) receiving different levels of nitrogen addition (18th June 02). (a) 0 Kg N ha^{-1} y^{-1}, (b) 10 Kg N ha^{-1} y^{-1}, (c) 20 Kg N ha^{-1} y^{-1}, (d) 50 Kg N ha^{-1} y^{-1}. n=6.

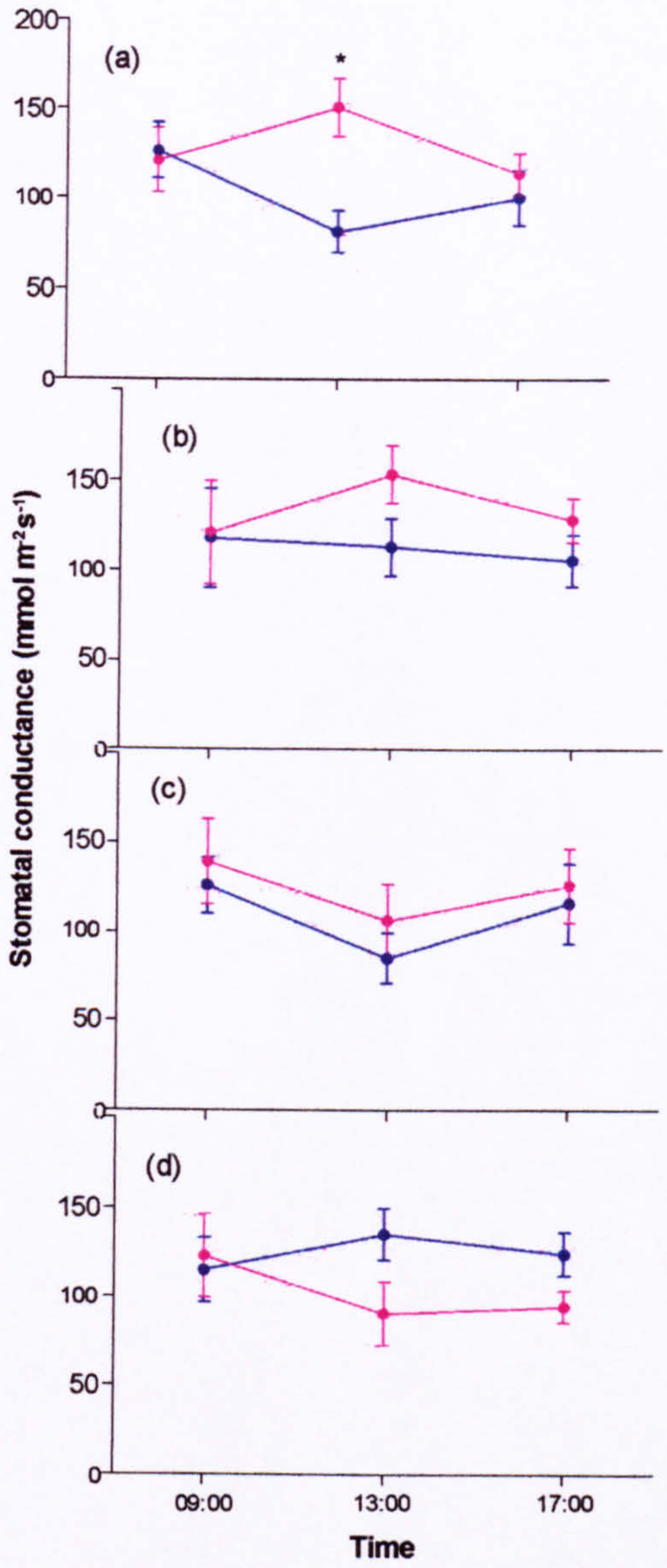


Figure 5.2 Stomatal conductance (mean ± SE) in *Ligustrum ovalifolium* plants in CFA (●) and exhaust gas-polluted air (●; 100 ppb NO_x) receiving different levels of nitrogen addition (17th June 02). Oneway ANOVA were performed for each time point. Asterisks denote the probability of difference between CF and exhaust gas-polluted plants (* $p<0.05$). (a) 0 Kg N ha^{-1} y^{-1}, (b) 10 Kg N ha^{-1} y^{-1}, (c) 20 Kg N ha^{-1} y^{-1}, (d) 50 Kg N ha^{-1} y^{-1}. n=6.

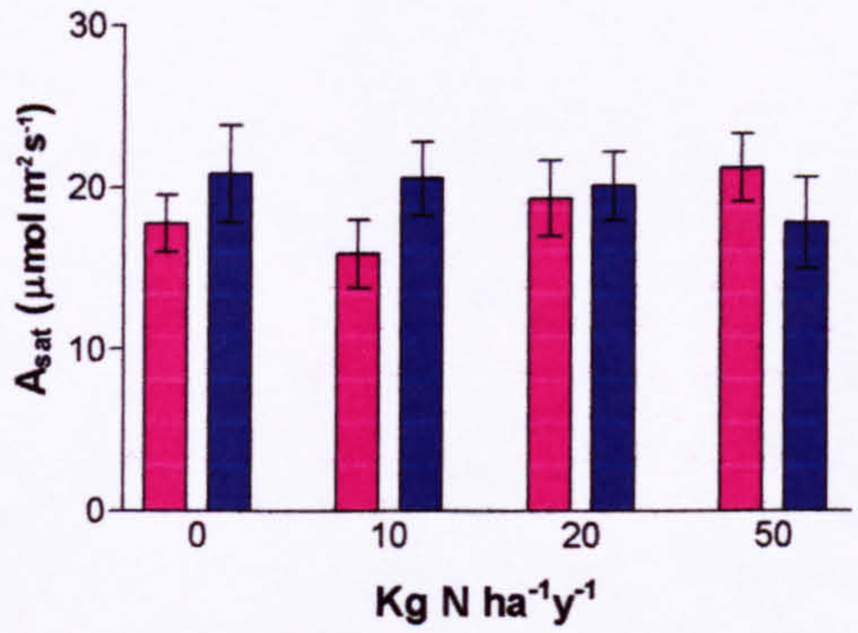


Figure 5.3 Assimilation (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (16-17th July 02). n=6.

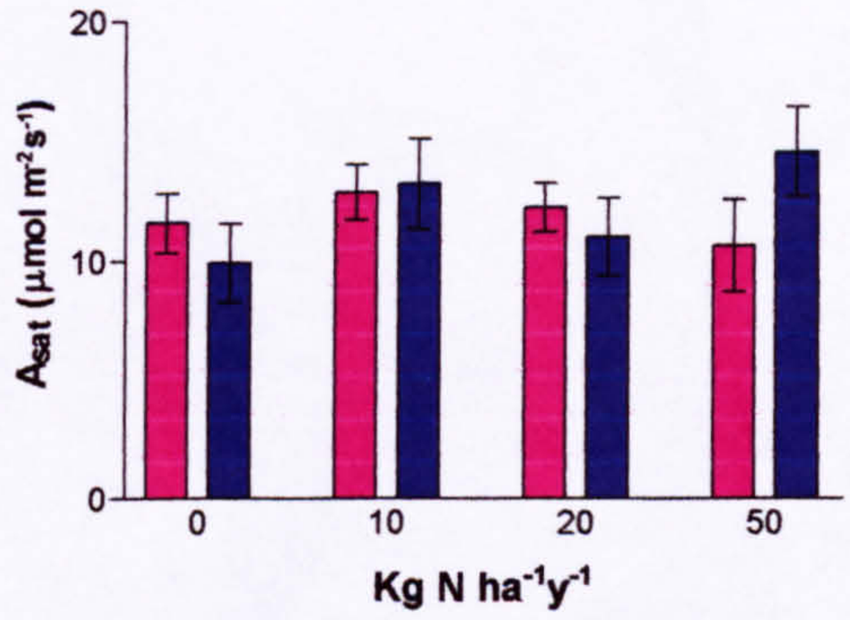


Figure 5.5 Assimilation (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th July 02). n=6.

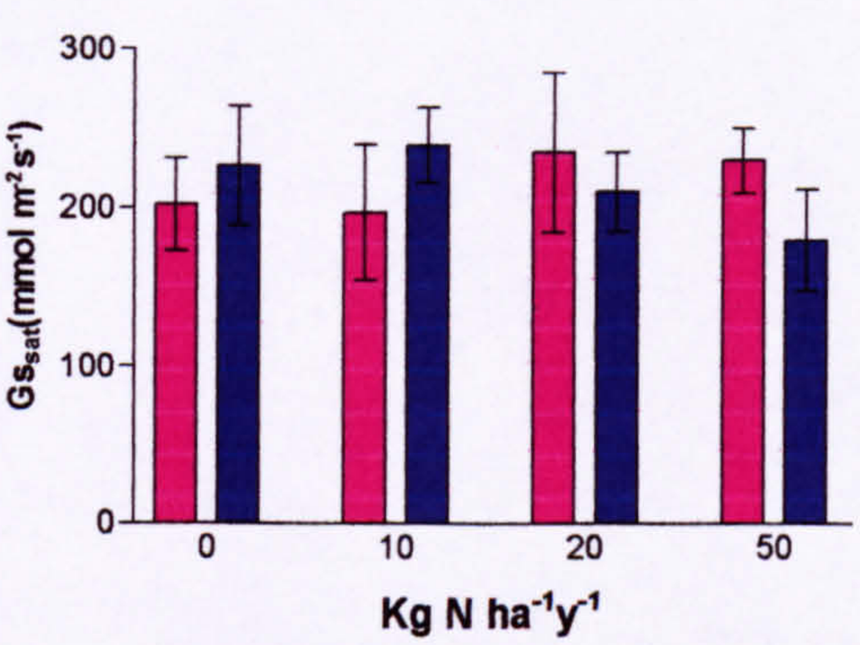


Figure 5.4 Stomatal conductance (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (16-17th July 02). n=6.

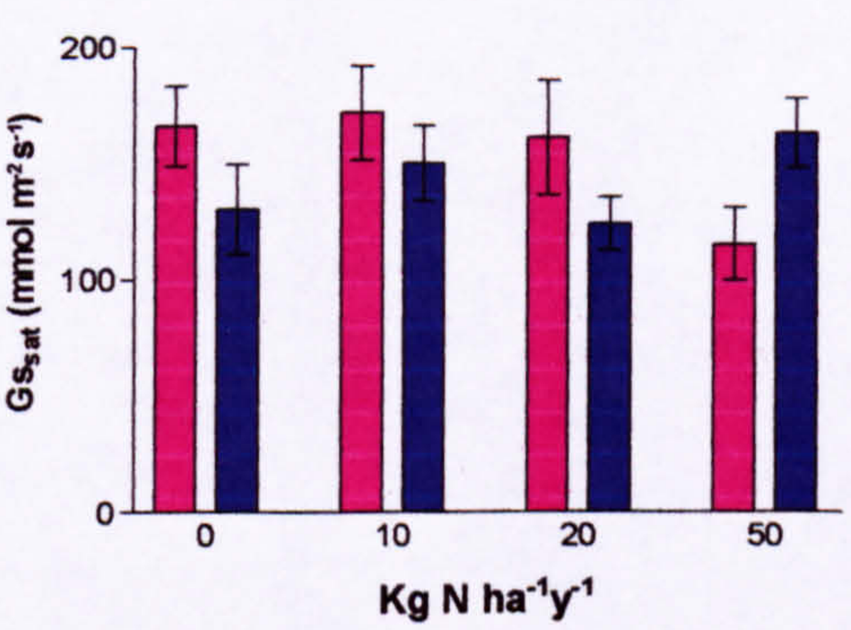


Figure 5.6 Stomatal conductance (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th July 02). n=6.

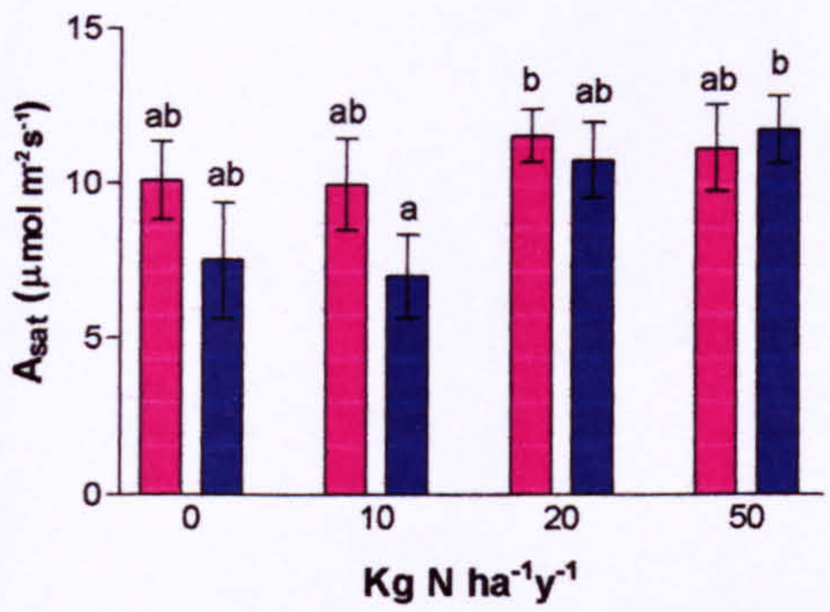


Figure 5.7 Assimilation (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (13-15th August 02). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6. Within-treatment effects were detected for exhaust gas polluted plants.

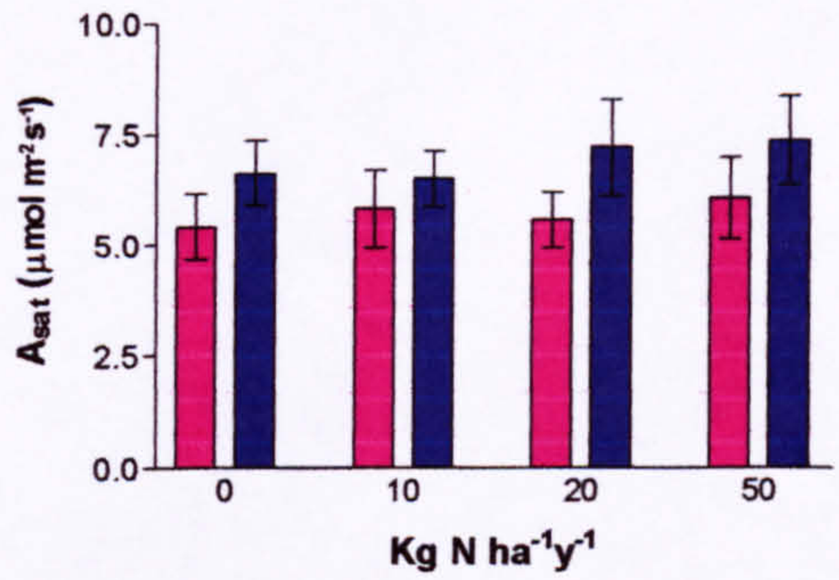


Figure 5.9 Assimilation (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th August 02). n=6.

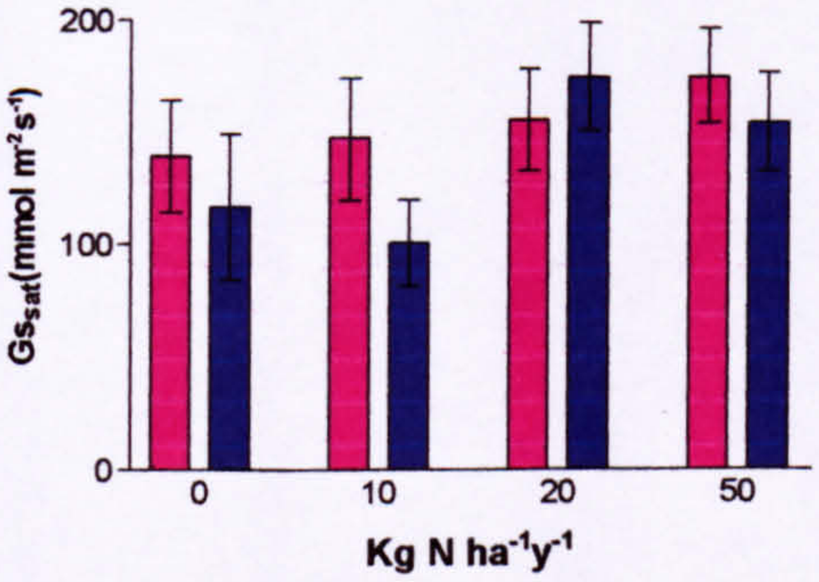


Figure 5.8 Stomatal conductance (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (13-15th August 02). n=6.

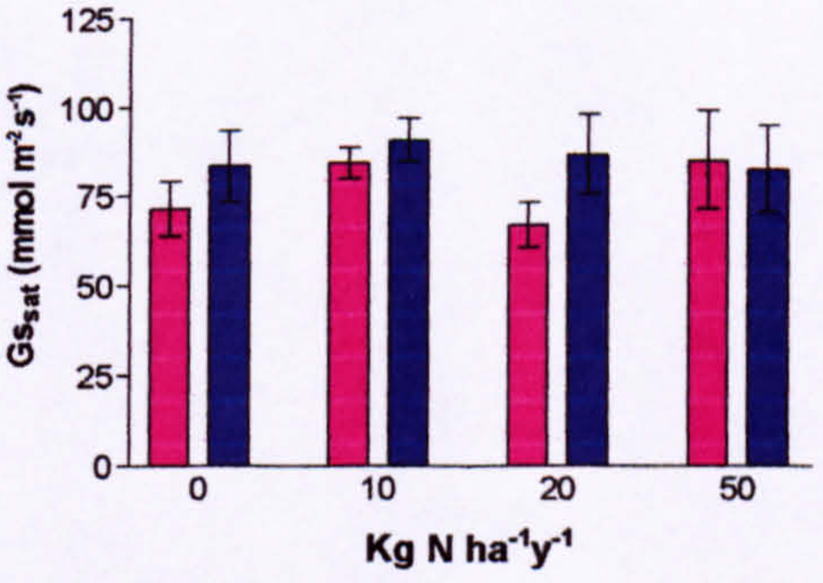


Figure 5.10 Stomatal conductance (mean ± SE) at saturated light (800 µmol m^{-2} s^{-1}) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th August 02). n=6.

5.3.3 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were carried out simultaneously to measurements of A_{sat} and Gs_{sat}, on 13-19 August 2002. In *Cornus sanguinea*, no effects of pollution or nitrogen treatment were evident (Figures 5.11 and 5.12). In

Ligustrum ovalifolium, exhaust gas pollution had a significant overall effect of increasing Fv/Fm compared with plants in CFA (twoway ANOVA, Appendix 51; $p=0.021$ for Fv/Fm; $p<0.001$ for AREA). The data are plotted in Figures 5.13 and 5.14. Both Fv/Fm and AREA values followed a similar pattern, tending to decrease with increasing soil N addition in CFA. In exhaust gas-polluted air, values tended to increase with increasing soil N, although the effect of nitrogen treatment was not significant.

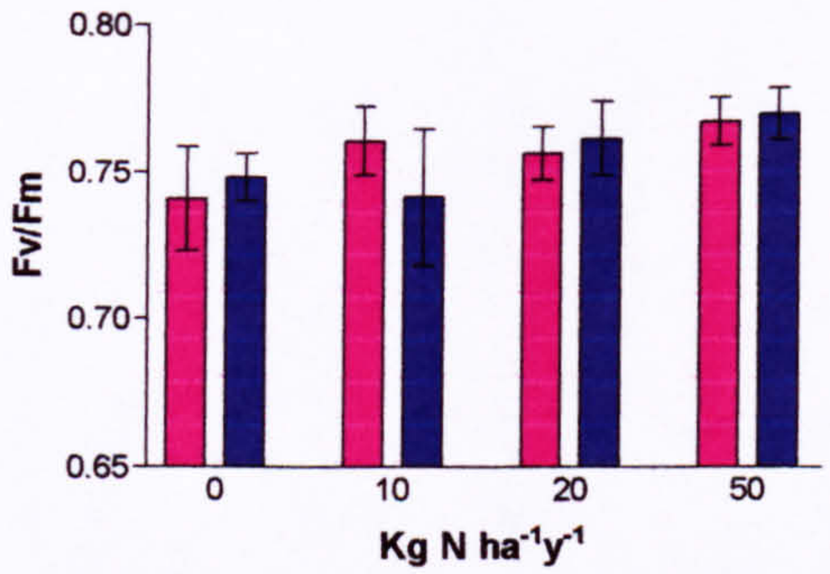


Figure 5.11 Fv/Fm values (mean ± SE) for *Cornus sanguinea* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x) under different levels of nitrogen addition (13-15th August 02). n=8.

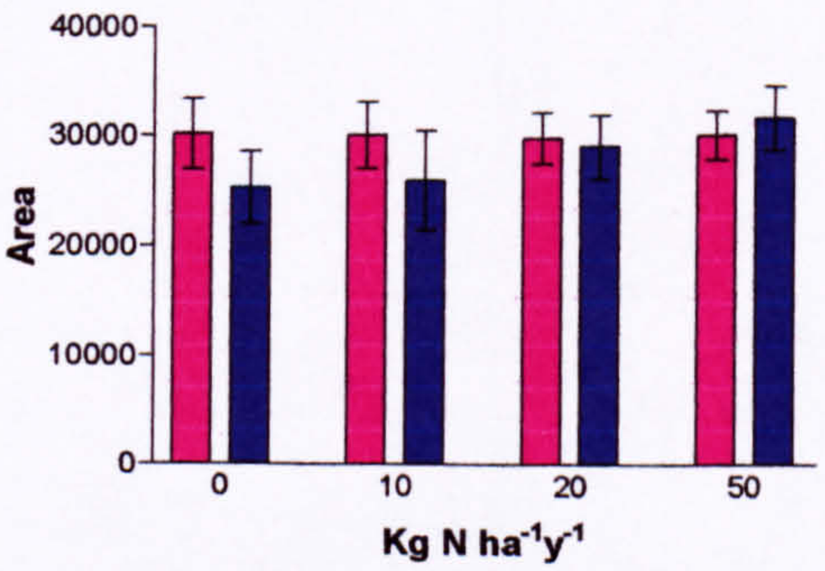


Figure 5.12 AREA values (mean ± SE) for *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x) under different levels of nitrogen addition. (13-15th August 02). n=8.

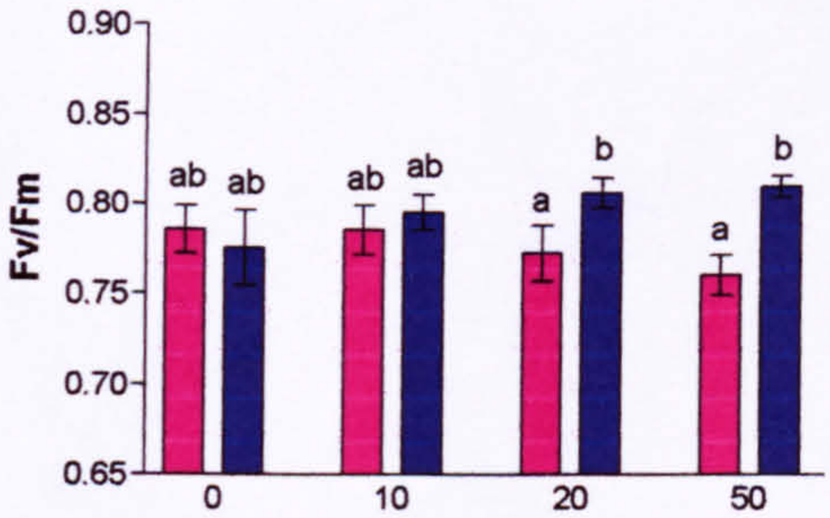


Figure 5.13 Fv/Fm (mean ± SE) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th August 02). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=8.

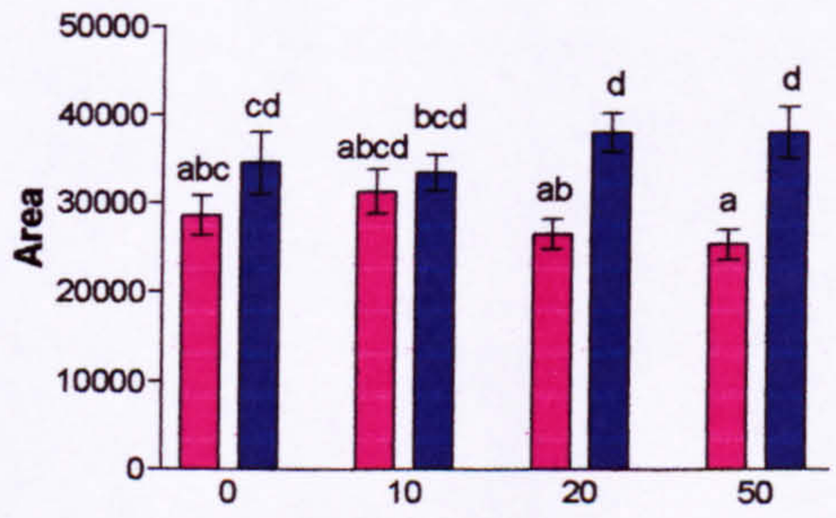


Figure 5.14 AREA values (mean ± SE) in *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (18-19th August 02). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=8.

5.3.4 Nitrate reductase activity

Exhaust gas pollution and nitrogen treatment both had significant (non-interactive) effects on leaf NR activity in *Cornus sanguinea* (twoway ANOVA, Appendix 52; p=0.003 for pollution; p=0.033 for nitrogen treatment). The pollution tended to stimulate higher foliar activities of the enzyme, while the level of nitrogen addition tended to decrease it (Figure 5.15). NR activity in the roots was not affected by either pollution or nitrogen treatments (Figure 5.16).

Exhaust gas pollution also had a stimulatory effect on NR activity in *Ligustrum ovalifolium* leaves (twoway ANOVA, Appendix 53; $p<0.001$), but nitrogen

addition had no effect. In the roots, NR activity was significantly influenced by both pollution (twoway ANOVA, Appendix 53; $p=0.035$) and nitrogen addition ($p<0.001$). Both had the effect of suppressing the activity of the enzyme (Figures 5.17 and 5.18).

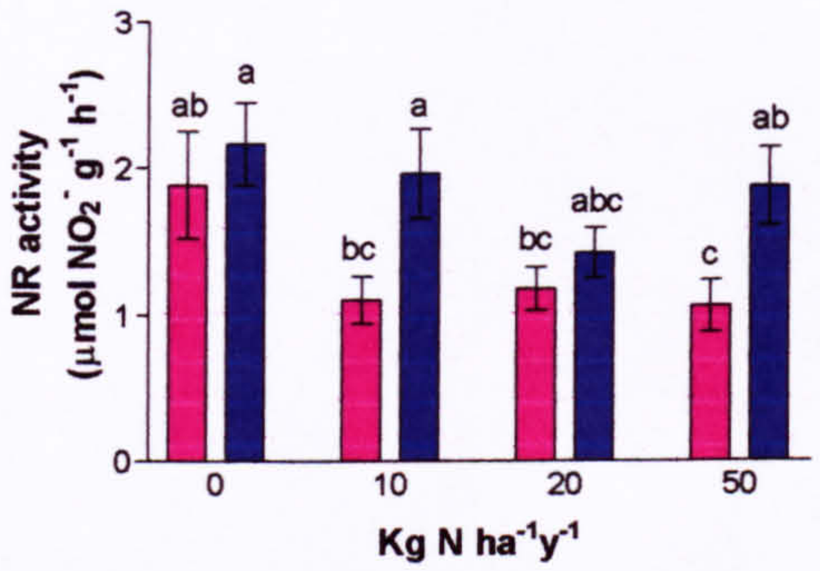


Figure 5.15 Nitrate reductase activity in leaves of *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.

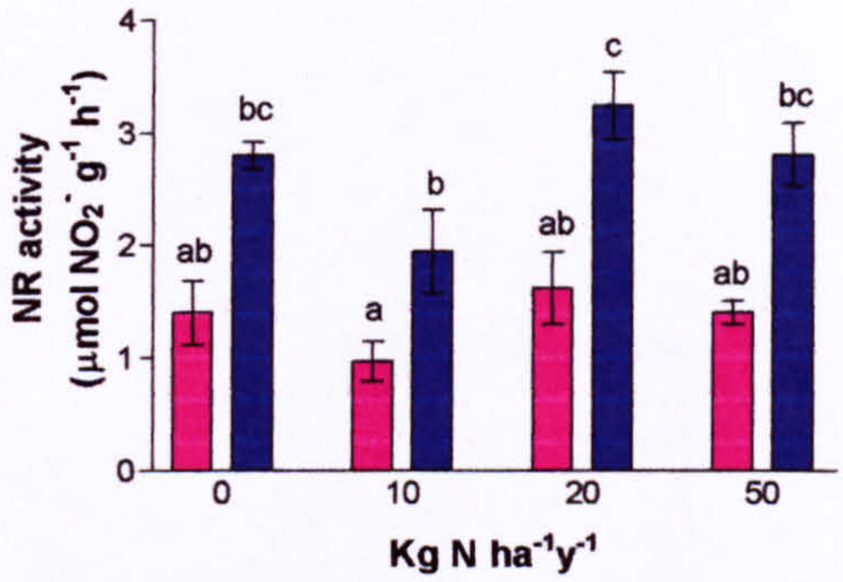


Figure 5.17 Nitrate reductase activity in leaves of *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.

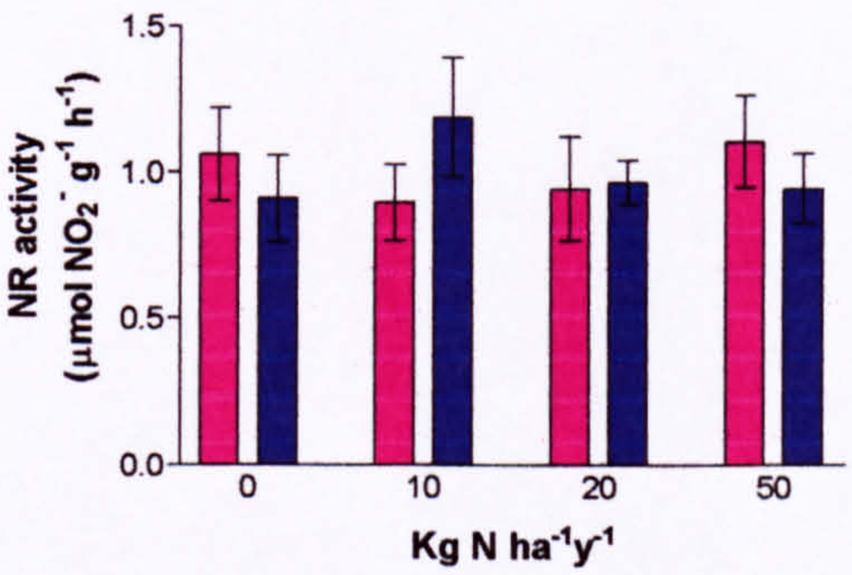


Figure 5.16 Nitrate reductase activity in roots of *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). n=6.

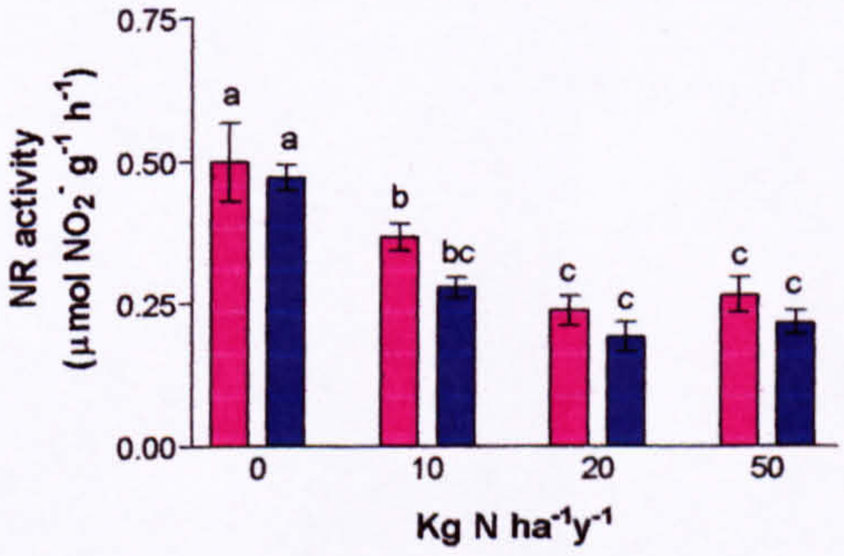


Figure 5.18 Nitrate reductase activity in roots of *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.

5.3.5 Foliar nitrogen concentration

For *Cornus sanguinea*, there was not enough leaf material available for nitrogen analysis at the end of the season, and so samples were obtained from material collected from the Solardomes during 2001 (well-watered plants from the drought experiment). Figure 5.19 shows the percentage nitrogen in leaves of *Cornus sanguinea* plants in CFA and exhaust gas-polluted air. There was no difference in nitrogen content between pollution treatments. In *Ligustrum ovalifolium*, both the pollution treatment and the nitrogen treatment were compared (Figure 5.20). Neither pollution nor nitrogen had an overall effect on nitrogen content, but at the highest nitrogen addition treatment (50 Kg N ha^{-1} y^{-1}), plants exposed to exhaust gas pollution had significantly greater percentage nitrogen compared with those in CFA ($p<0.05$). These were the plants receiving the greatest combined input of nitrogen from both the atmosphere and the soil.

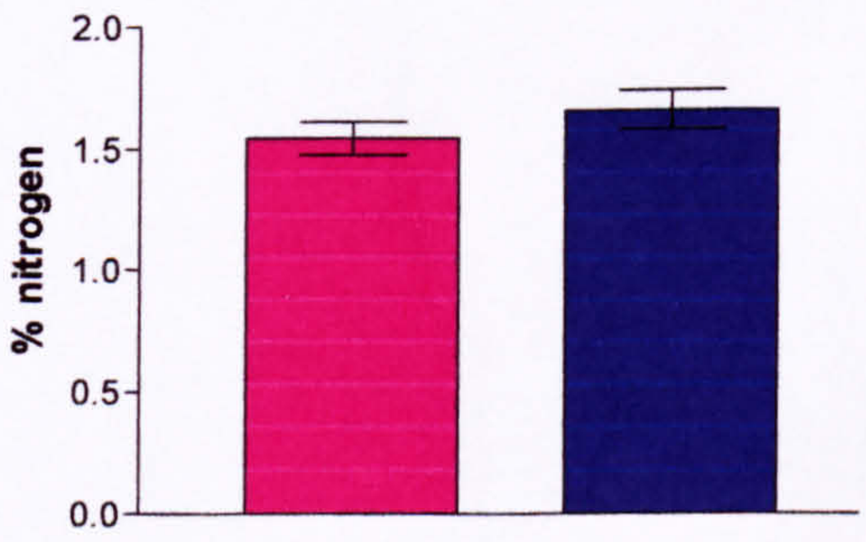


Figure 5.19 Percent nitrogen in the leaves of *Cornus sanguinea* in plants CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). n=4.

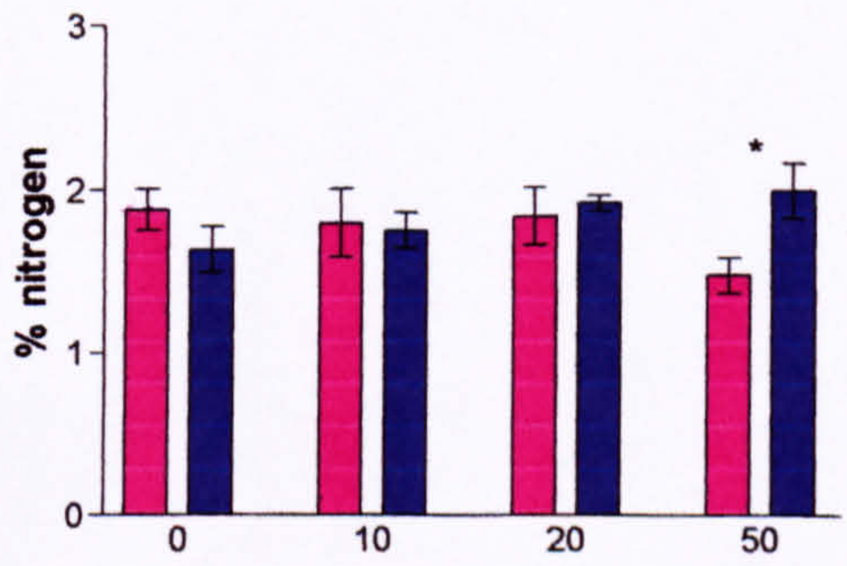


Figure 5.20 Percent nitrogen in the leaves of *Ligustrum ovalifolium* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Astericks denote probability of difference between CFA and exhaust gas-polluted air (Oneway ANOVA with Duncan's multiple test range; * $p<0.05$). n=6.

5.3.6 Growth

Graphs showing increase in plant height over the course of the fumigation are given in Figures 5.21 and 5.22. In both *Cornus sanguinea*, and *Ligustrum ovalifolium*, exhaust gas pollution and nitrogen addition had no significant effects on plant height (repeated measures ANOVAs; Appendices 54 and 55).

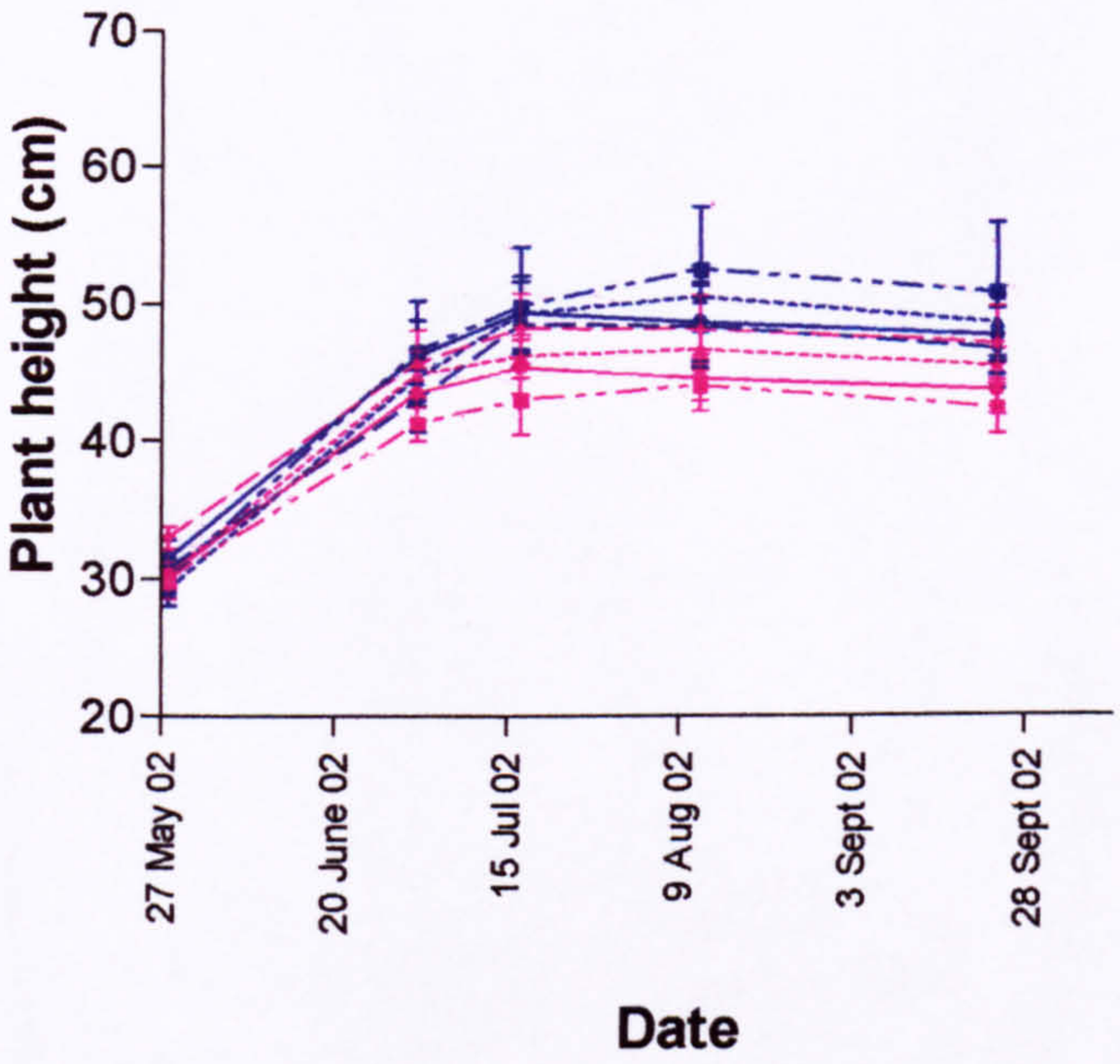


Figure 5.21 Increase in height of *Cornus sanguinea* plants in CFA (pink symbols) at 0 (●; solid line), 10 (▲; dotted line), 20 (♦; dashed line) and 50 (■; dotted and dashed line) Kg N ha^{-1} y^{-1}, and in exhaust gas-polluted air (blue symbols) at 0 (●; solid line), 10 (▲; dotted line), 20 (♦; dashed line) and 50 (■; dotted and dashed line) Kg N ha^{-1} y^{-1}. n=8.

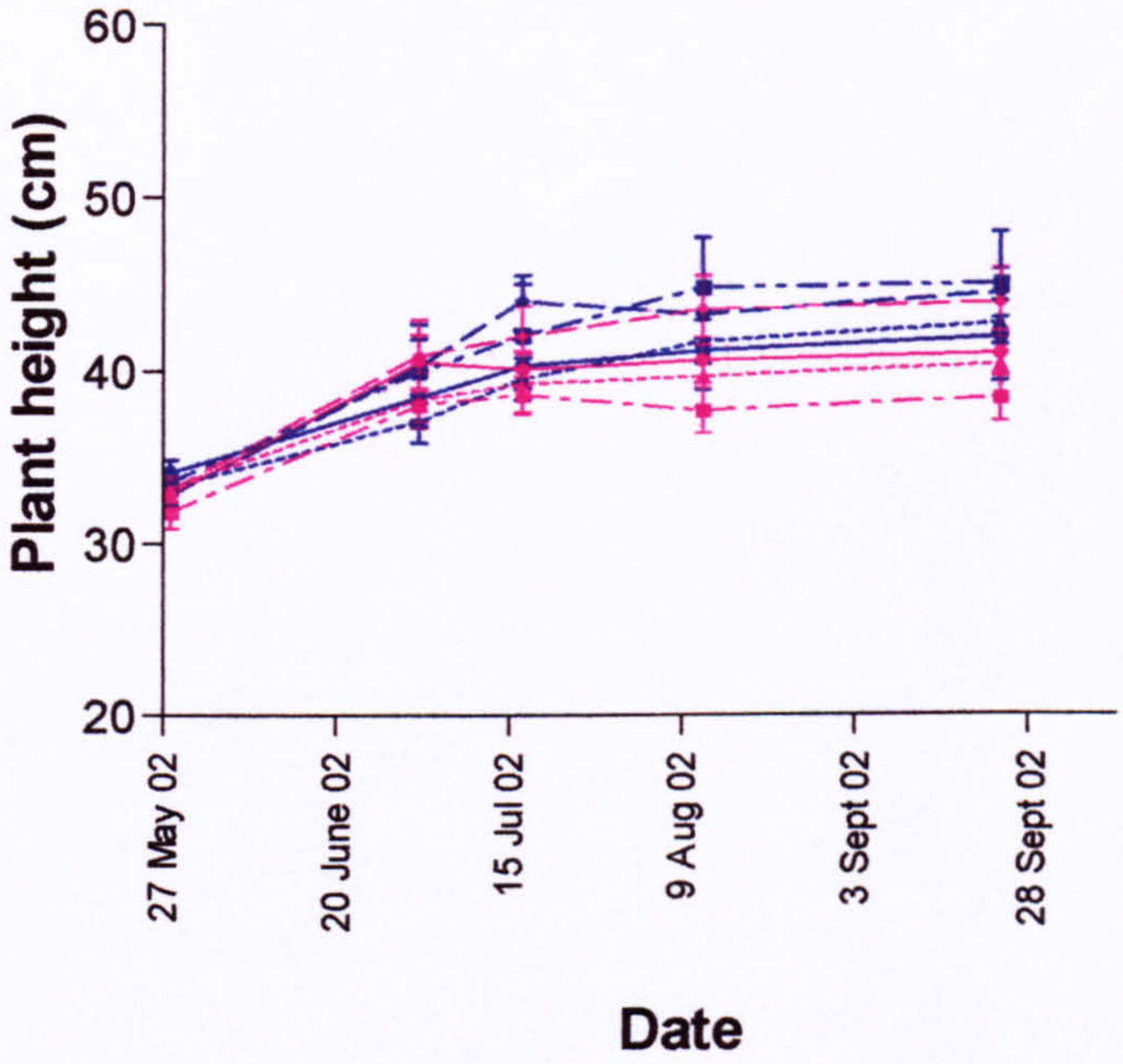
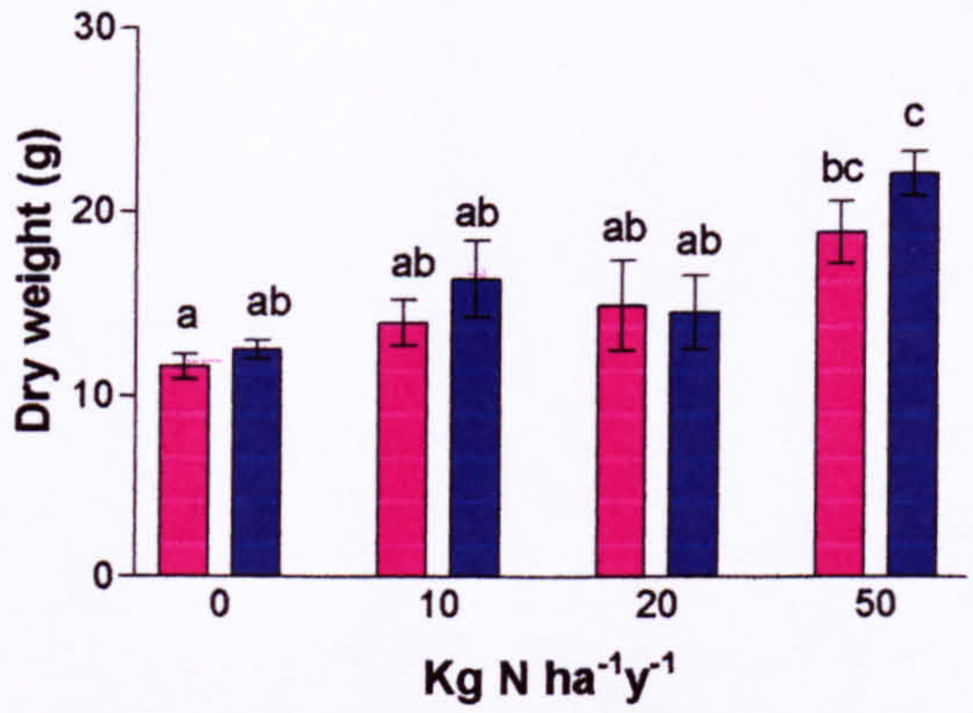


Figure 5.22 Increase in height of *Ligustrum ovalifolium* plants in CFA (pink symbols) at 0 (●; solid line), 10 (▲; dotted line), 20 (♦; dashed line) and 50 (■; dotted and dashed line) Kg N ha^{-1} y^{-1}, and in exhaust gas-polluted air (blue symbols) at 0 (●; solid line), 10 (▲; dotted line), 20 (♦; dashed line) and 50 (■; dotted and dashed line) Kg N ha^{-1} y^{-1}. n=8.

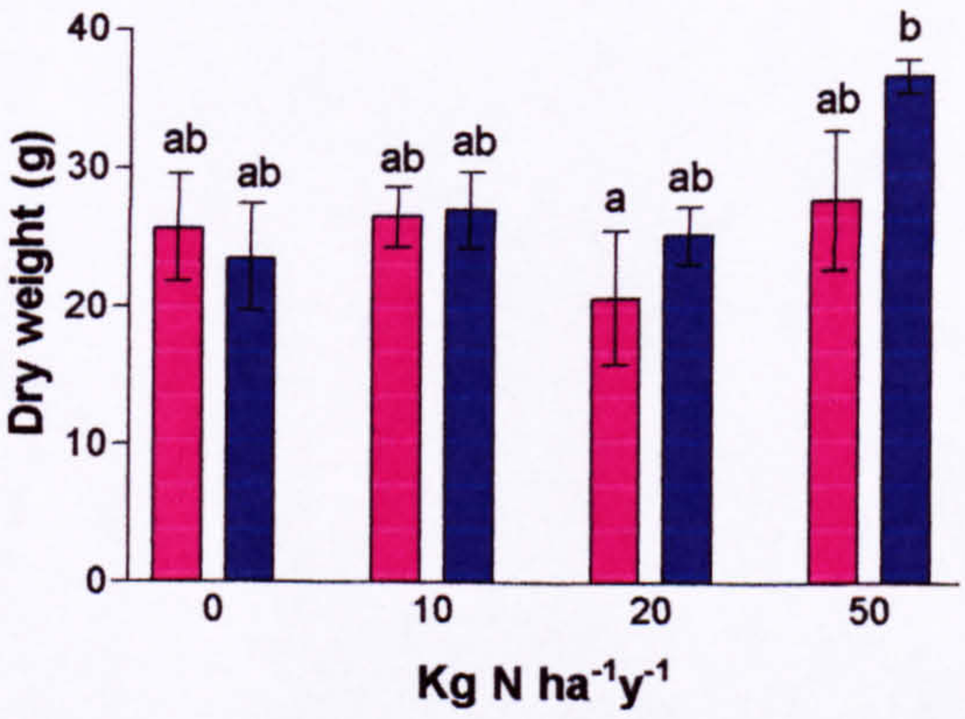
5.3.6 Biomass

Dry weights of the woody stems of *Cornus sanguinea* at the end of the season were significantly affected by nitrogen addition, with plants in higher nitrogen treatments having greater dry weights (twoway ANOVA, appendix 56; p=0.001; Figure 5.23). Exhaust gas pollution did not influence root dry weight, except in the highest N addition treatment, where plants in exhaust gas-polluted air had significantly ($p<0.05$) greater root dry weight compared with those in CFA (Figure 5.24). Mean R:S appeared to show a downward trend in plants in CFA, but not in plants from exhaust gas-polluted air (Figure 5.25).

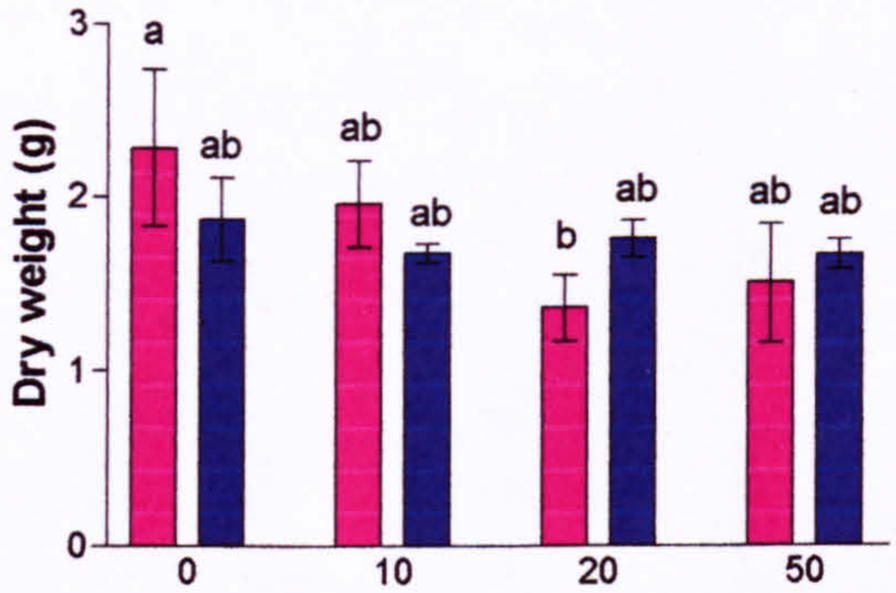
In *Ligustrum ovalifolium*, pollution and nitrogen addition had no significant effects on dry weights of shoots or roots (Figures 5.26 and 5.27). In the highest nitrogen addition treatment (50 Kg N ha^{-1} y^{-1}), the mean R:S of plants in exhaust gas-polluted air was higher compared with those in CFA (Figure 5.28). This effect was not seen in the other nitrogen addition treatments.



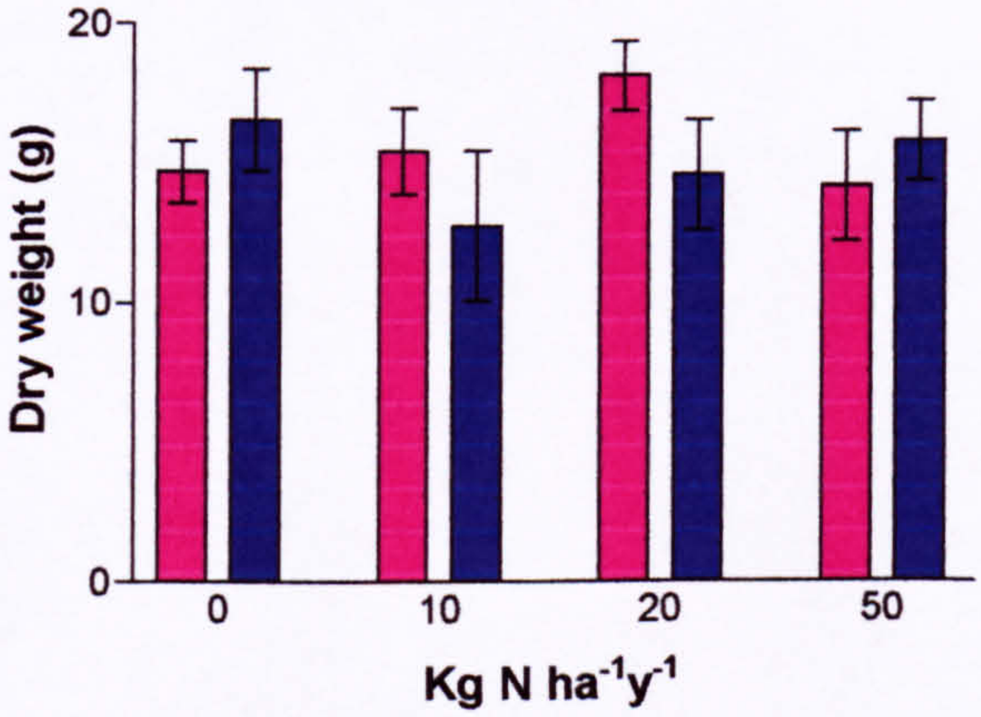
5.23 Dry weight of woody stems of *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.



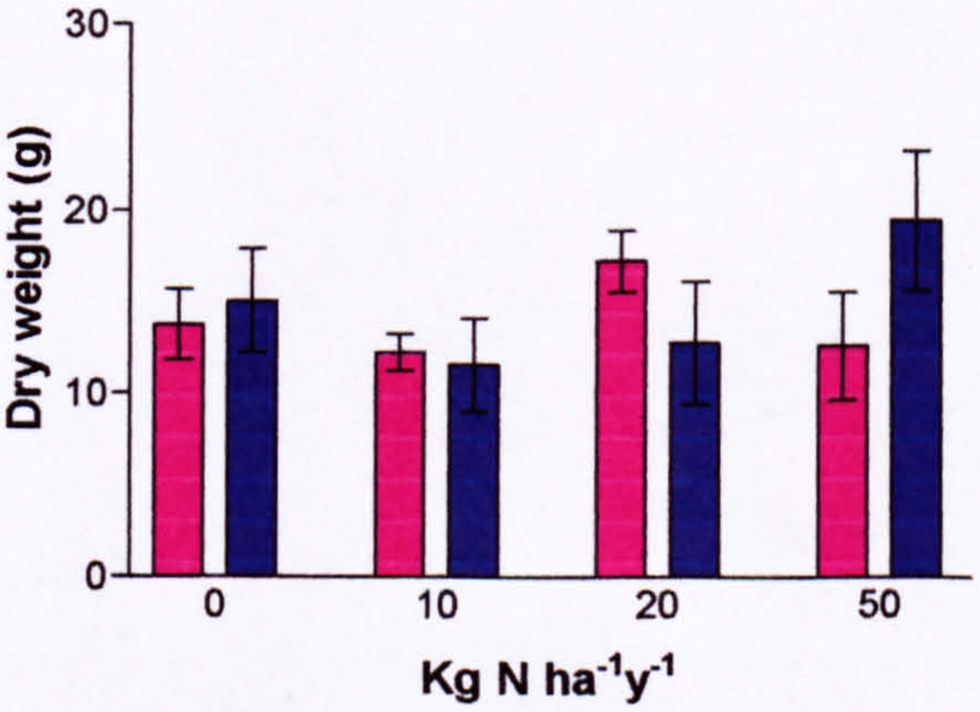
5.24 Dry weights of roots of *Cornus sanguinea* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.



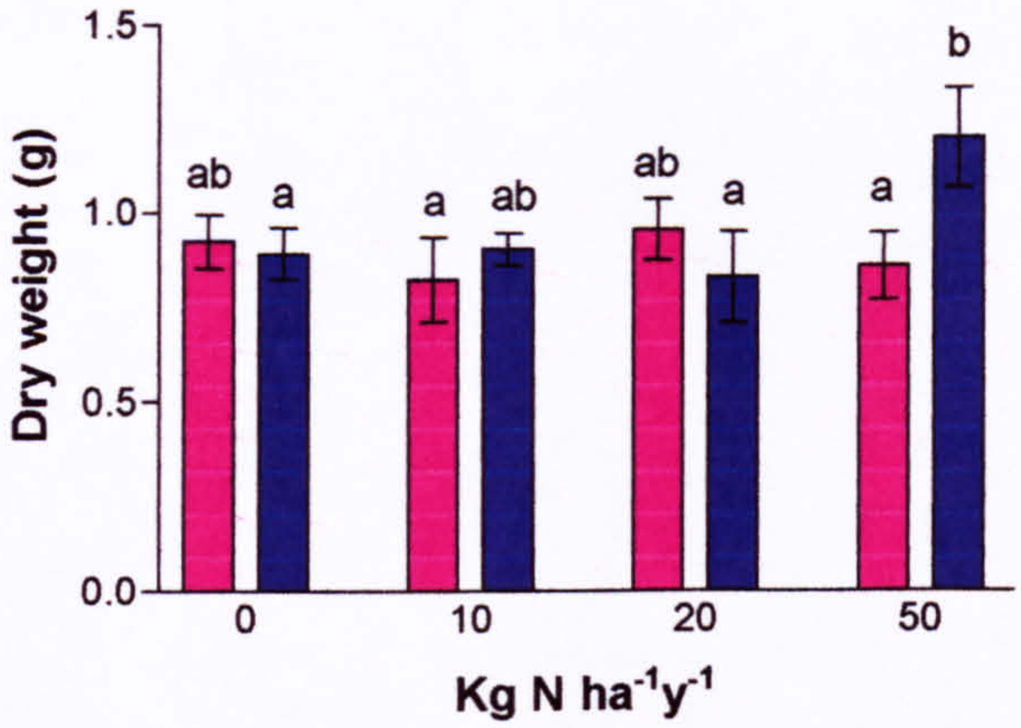
5.25 Root : shoot ratio of *Cornus sanguinea* based on dry weights in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.



5.26 Dry weight of shoots of *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). n=6.



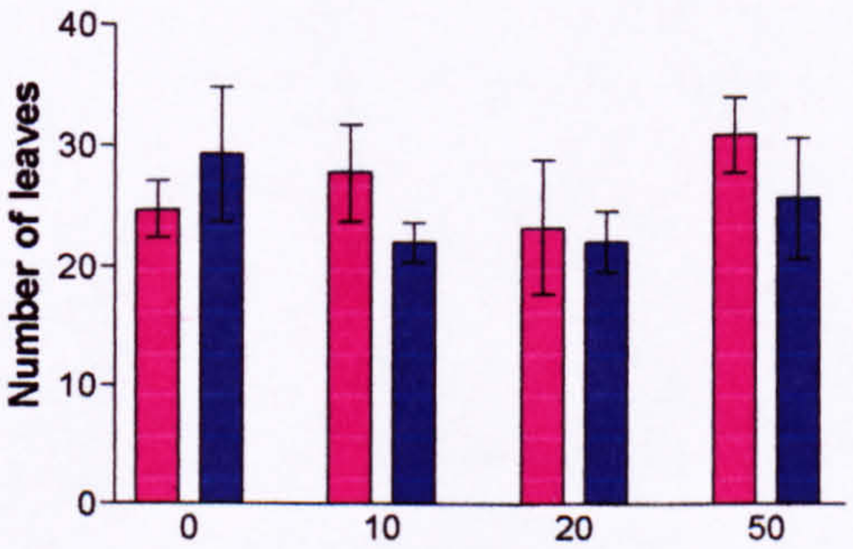
5.27 Dry weights of roots of *Ligustrum ovalifolium* in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). n=6.



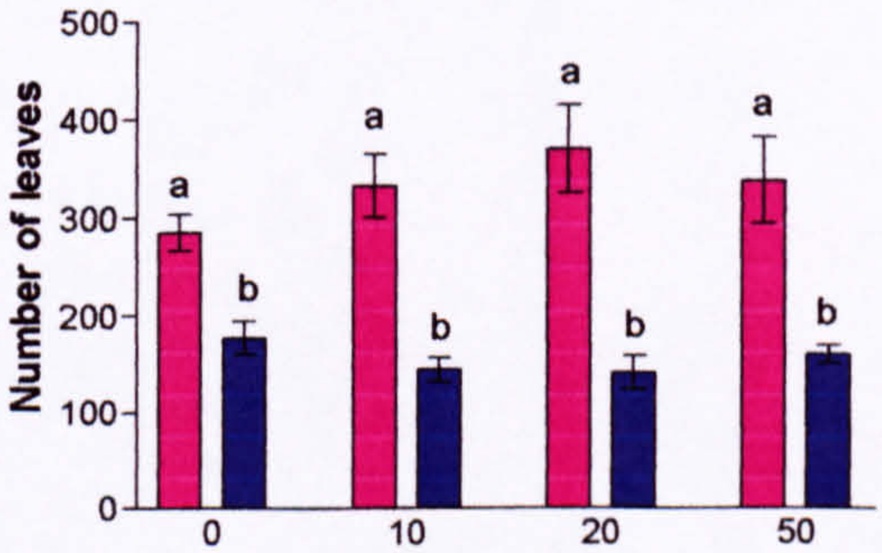
5.28 Root : shoot ratio of *Ligustrum ovalifolium* based on dry weights in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=6.

5.3.7 Leaf retention near the end of the growing season

In *Cornus sanguinea*, neither pollution nor nitrogen addition altered the timing of leaf fall compared with controls (Figure 5.29). In *Ligustrum ovalifolium*, however, exhaust gas pollution had a significant effect on leaf retention (twoway ANOVA, Appendix 57; $p<0.001$). Plants in polluted conditions retained fewer leaves near the end of the growing season (24 September 2002) compared with those in CFA (Figure 5.30).



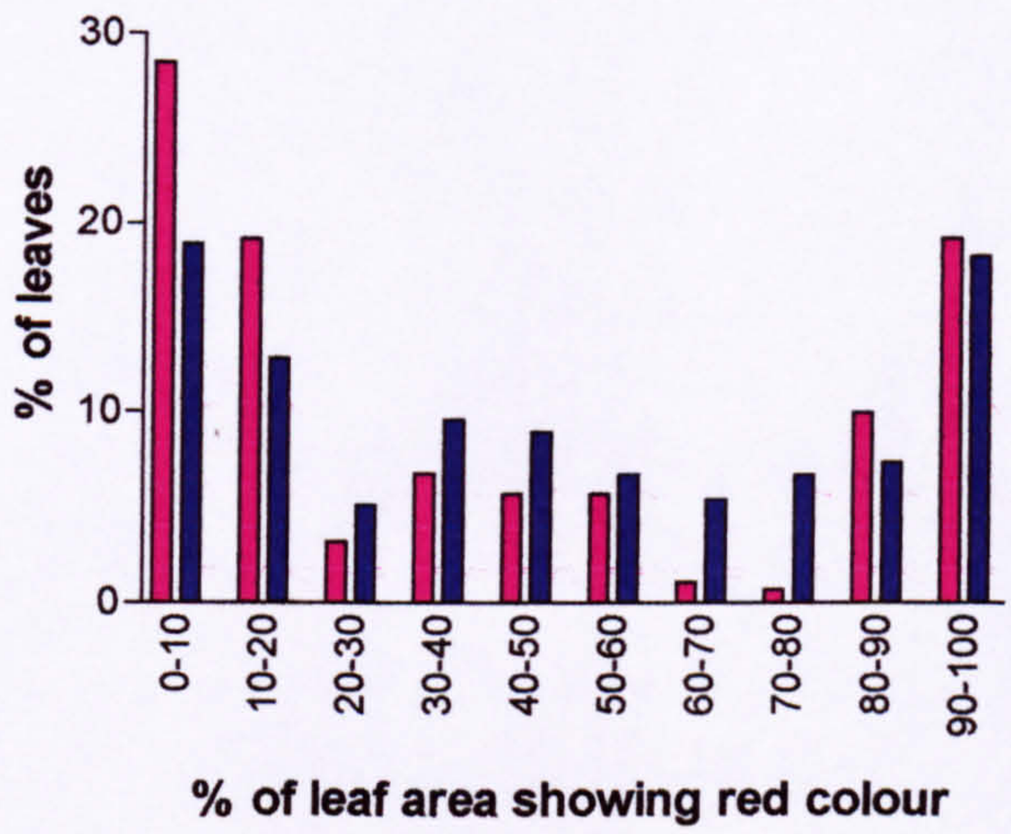
5.29 Number of leaves remaining on *Cornus sanguinea* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x) near the end of the season. n=8.



5.30 Number of leaves remaining on *Ligustrum ovalifolium* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x) near the end of the season. Data were subjected to oneway ANOVA and Duncan's multiple range test. Different letters indicate significant ($p<0.05$) differences between means. n=8.

5.3.8 Senescence in *Cornus sanguinea* determined by leaf colour

The percentage frequency of leaves from exhaust gas-polluted air and CFA (from the 0 Kg N h^{-1} y^{-1} treatment) falling into each category of % red coloration are given in Figure 5.31. The absolute values falling into each category were used to perform a χ^2 test (Appendix 58), which showed that there was a significant ($p<0.001$) effect of pollution on leaf pigmentation. CFA plants had more leaves with 0-20% of the leaves covered by red pigmentation compared with plants in exhaust gas-polluted air. Plants in CFA had less leaves falling into the categories between 40-80% red coloration compared with plants from exhaust gas-polluted air.

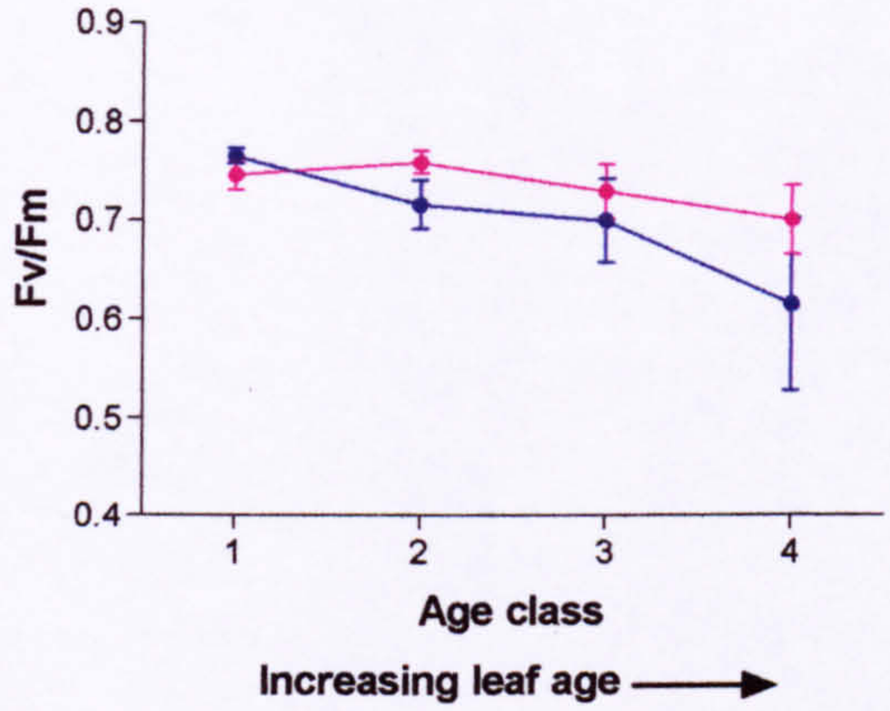


5.31 Percentage frequency of leaves in different stages of senescence in *Cornus sanguinea* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x), determined by the percentage of the area of the leaf showing red coloration. Plants receiving 0 Kg N ha^{-1} y^{-1} from clean air and exhaust gas-polluted air are compared. A χ^2 =test showed significant ($p<0.001$) difference between treatments.

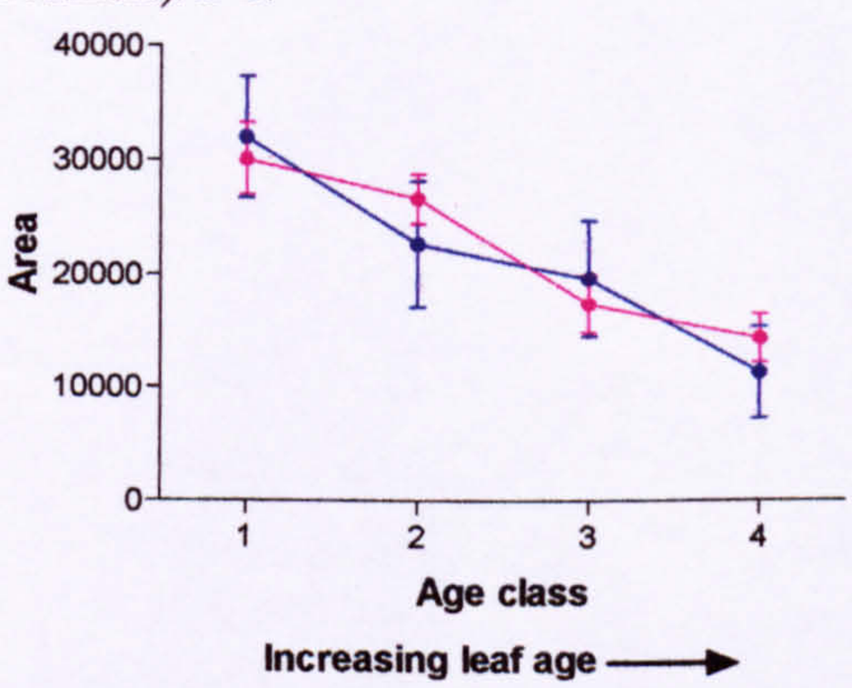
5.3.9 Senescence in *Cornus sanguinea* determined by chlorophyll fluorescence

Values of Fv/Fm and AREA for leaves of different age classes from exhaust gas-polluted air and CFA (from the 0 Kg N h^{-1} y^{-1} treatment) are given in Figures

5.32 and 5.33. Neither pollution nor leaf age had any effect on Fv/Fm values (twoway ANOVA, Appendix 59; Figure 5.32). AREA values were significantly influenced by leaf age (Appendix 59; p=0.003), but not by pollution. There was a decrease in AREA values with increasing leaf age (Figure 5.33).



5.32 Fv/Fm of leaves of different ages in *Cornus sanguinea* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (plants from the 0 Kg N ha^{-1} y^{-1} treatment). n=6.



5.33 Area values of leaves of different ages in *Cornus sanguinea* plants in CFA (■) and exhaust gas-polluted air (■; 100 ppb NO_x). (plants from the 0 Kg N ha^{-1} y^{-1} treatment). n=6.

5.4 Discussion

In urban situations there is a cumulative deposition of nitrogen to the soil from vehicle emissions and other anthropogenic sources. For plants this is coupled

with the presence of gaseous nitrogenous pollutants. Exposure to enhanced soil and atmospheric nitrogen could interact to cause alterations in plant responses.

In *Cornus sanguinea*, exhaust gas pollution and nitrogen addition did not affect plant height (Figure 5.21). Increasing levels of nitrogen addition did, however, increase stem dry weight (Figure 5.23). The same effect has been observed in conifers, where fertilization with mineral nitrogen leads to an increase in shoot growth and wood production (e.g. Thomas and Mead, 1992). In the present study, R:S of *Cornus sanguinea* appeared to show a downward trend with increasing nitrogen deposition in CFA plants, but there was no such trend in plants in exhaust gas-polluted air (Figure 5.25).

When nitrogen becomes more available to the roots, the R:S ratio is expected to decrease, since less root area per unit shoot area is required for nutrient uptake (e.g. George and Seith, 1998). This response represents the reverse of the situation where a shift in biomass allocation is encouraged to structures responsible for the acquisition of a limiting resource (e.g. Brouwer, 1962). Similar shifts in R:S have been reported in several annual and forest tree species in response to differing levels of nitrogen availability (reviewed in George and Seith, 1998). Although this shift in R:S is a "logical" response by the plant in terms of nitrogen availability, it can have negative consequences, for example on drought resistance capabilities (e.g. Gordon *et al.*, 1999).

The absence of this effect, of decreasing R:S with increasing root nitrogen supply, in the plants growing in exhaust gas-polluted air could be a consequence of the exhaust gas pollution making nitrogen available for shoot uptake. Foliar nitrogen uptake was indeed enhanced in the leaves of *Cornus sanguinea* growing in exhaust gas-polluted air, as reflected by their significantly enhanced NR activity compared with plants in CFA (Figure 5.15). This was not reflected in the nitrogen content of the leaves (Figure 5.19). Exhaust gas pollution and nitrogen addition did not have any influence on root NR activity in this species.

Neither exhaust gas pollution nor nitrogen deposition had any marked influence on plant height (Figure 5.22) or dry weights of plant parts (Figures 5.26 and 5.27) in *Ligustrum ovalifolium*. There was no alteration in R:S with pollution, except that in the highest nitrogen addition treatment (50 Kg N ha^{-1} y^{-1}), plants in exhaust gas-polluted air had significantly higher R:S compared with those in CFA (Figure 5.28). This is opposite to the expected response to increased availability of nitrogen, and the cause of this is not known.

In *Ligustrum ovalifolium* plants, there was again an up-regulation of NR activity in the leaves in response to exhaust gas pollution, but no effect of nitrogen deposition (Figure 5.17). At the highest level of nitrogen addition, this was reflected in increased concentrations of foliar nitrogen (Figure 5.20). This implies that the NO_2 taken up from the atmosphere was incorporated into the leaves, as has been found in several studies of plants at the roadside (e.g. Port and Thompson, 1980; Spencer and Port, 1988; Spencer *et al.*, 1988). This effect was only seen in plants in the highest soil nitrogen treatment, the plants receiving the greatest nitrogen input from both the atmosphere and the soil. Increased nitrogen content in the leaves could represent altered nutritional quality and has the potential to influence plant-pest interactions by increasing the availability of nitrogen for herbivores (e.g. Riemer and Whittaker, 1989). NR activity in the roots was slightly decreased by exhaust gas pollution, and greatly decreased by increasing soil nitrogen deposition (Figure 5.18). This latter response is as expected, since a greater supply of nitrite ions reduces the need for their production by the enzyme (Mansfield and Lucas, 1996).

In previous experiments (Chapter 4), both of the study species were found to have lower stomatal conductance in exhaust gas-polluted air compared with CFA. In the present study, there was no stomatal response of *Cornus sanguinea*. There are several possible explanations for this change in response between studies in 2001 (Chapter 4) and those in the present study (2002). The change in

the exhaust gas fumigation system between experimental seasons altered the proportions of the gases in the pollution mixture. Although the overall NO_x concentration was similar in both years (close to 100 ppb), the NO:NO_2 ratio changed from 1.44 in 2001 to 1.94 in 2002 (Chapter 2; Table 2.1). Concentrations of benzene increased slightly between 2001 and 2002, and those of toluene increased dramatically (from an average of 0.9 ng l-1 in 2001 to 4.8 ng l-1 in 2002; Table 2.1). The timing of onset of exhaust gas exposure and the timing of measurements was also different between the two years (Table 2.4). Stomatal response has already been shown to alter over the course of the growing season (Chapter 4). In *Ligustrum ovalifolium*, conductance was suppressed under exhaust gas exposure in plants receiving no additional nitrogen (Figure 5.2). This result is in agreement with previous findings (Chapter 4).

Photosynthesis was unaffected by both exhaust gas pollution and nitrogen addition in July in both species. By August, assimilation had dropped by almost 50% compared with July values, probably due to a decline in growth. In *Cornus sanguinea*, this decrease was more marked in plants in exhaust gas-polluted air, but only in the two lowest nitrogen treatments (0 and 10 Kg N ha^{-1} y^{-1}) (Figure 5.7). This drop in carbon acquisition might be expected to have consequences for growth. However, plant height (Figure 5.21) and above-ground dry weight (Figure 5.23) were found to be unaffected by exhaust gas pollution in this species. Any effects on photosynthesis were not reflected in chlorophyll fluorescence parameters.

In *Ligustrum ovalifolium*, no effects of the pollution or nitrogen treatment on A_{sat} were found in either July or August. Pollution did, however have an overall effect of increasing both Fv/Fm and AREA values in this species (Figures 5.13 and 5.14). The Fv/Fm values of all the plants fell below the optimal value of 0.83 measured for most species (e.g. Björkman and Demmig, 1987), but plants in exhaust gas-polluted air had a greater quantum yield of PSII compared with those in CFA. AREA values, which seem to be an even more sensitive indicator

of plant photosynthetic performance, showed a slight downward trend in CFA plants as the level of nitrogen addition increased. There was an opposite, upward trend with increasing nitrogen addition in plants in exhaust gas-polluted air, although the overall effect of nitrogen deposition was not significant. There are no published data on the effects of air pollution on AREA values, but Davison (personal communication) has found that in *Rudbeckia laciniata*, AREA decreased with leaf age, and was lower in plants injured by O_3 compared with uninjured plants. AREA might be useful as an indicator of pollution damage, provided it is measured in leaves of comparable age.

The stimulatory effect observed in *Ligustrum ovalifolium* of the pollution on the quantum yield of PSII, as measured by Fv/Fm and AREA values, is surprising. Other pollutants, such as O_3, have been found to *decrease* photosynthetic efficiency (e.g. Clark *et al.*, 2000b working with beech). Also, Scots pine trees growing in urban sites with high rates of traffic emissions were found to have lower Fv/Fm values compared with those in less polluted areas (Saarinen, 1993). A positive effect of urban pollution on these parameters might infer that *Ligustrum ovalifolium* is particularly resistant to urban pollution mixtures. Indeed, this species suffered fewer negative responses to the pollution compared with *Cornus sanguinea*.

The progression of normal foliar senescence appeared to be altered by exhaust gas pollution in both species, but not by the level of nitrogen addition. In *Ligustrum ovalifolium*, leaf fall was accelerated in plants growing in exhaust gas-polluted air compared with those in CFA (Figure 5.30). The final dry weights of the shoots were not influenced by exhaust gas pollution, but by the time the plants were harvested (several weeks after the leaf number measurements were taken), there were few leaves remaining on plants from either treatment. Although *Ligustrum ovalifolium* is normally an evergreen species, plants grown in the Solardomes, both in polluted air and CFA, did begin lose their foliage in the autumn. It is assumed that this was an effect of being grown in chambers. In

perennial deciduous plants, accelerated senescence might be expected to have consequences for performance in the following year, and for long-term health.

Although leaf number of *Cornus sanguinea* plants near the end of the season was not affected by exhaust gas pollution, the percentage cover of leaves by red pigmentation was significantly increased under polluted conditions compared with clean air controls (Figure 5.31). Acceleration of autumnal leaf colour change has also been found in beech in response to O_3 exposure, and there was a close correlation between colour and chlorophyll fluorescence (Mikkelsen and HeideJorgensen, 1996). The loss of green colour is one of the hallmarks of senescence, and in the case of red-senescing leaves, represents an accumulation of anthocyanins (e.g. in *Cornus stolonifera*; Field *et al.*, 2001). Anthocyanins are known to form a pigment layer in the palisade mesophyll that can decrease light capture by chloroplasts (Field *et al.*, 2001). This alteration in leaf colour did not however translate to an observable difference in the efficiency in PSII as measured by Fv/Fm or AREA values in *Cornus sanguinea*.

Ethylene is a plant hormone involved in many growth, aging and trophic responses, including premature leaf colour changes and shedding of leaves (Davison, 1974). This compound is a component of vehicle exhaust, and may have been the cause of the accelerated senescence observed in these species.

In terms of critical loads for nitrogen deposition to different vegetation types, commonly-planted urban shrubs such as those used in this experiment would be expected to fall into a low sensitivity class. Although temperate forest habitats are given a critical load range of 10 – 15 Kg N ha^{-1} y^{-1} (Bobbink *et al.*, 2002), this is based on ground flora, the most sensitive component of the ecosystem. The trees themselves are able to withstand greater deposition rates (up to 30 Kg N ha^{-1} y^{-1}) before showing a marked response (Kuylenstierna *et al.*, 1998). It is perhaps therefore not surprising that the effects of nitrogen deposition in the present study were quite subtle in many of the parameters measured. However,

there were effects of both exhaust gas pollution and nitrogen deposition on biomass partitioning, nitrate reductase activity and photosynthesis in *Cornus sanguinea* and on foliar senescence in both species. These alterations in plant functioning could have consequences for long-term health and stress avoidance capabilities.

Chapter 6: Effects of Urban Air Pollutants on Leaf Surface Characteristics

6.1 Introduction

6.1.1 Structure, function and composition of epicuticular waxes

The plant cuticle represents the largest aerial interface between plants and their environment (Kupčinskienė, 2001). Epicuticular waxes, often with a complex crystalline structure, cover or are embedded in the cuticle. They show great ultrastructural diversity throughout the plant kingdom, with wax sculpture forms including rods, plates, filaments, tubules, grains and ribbons. These surface structures range in size between 1 – 20 μm (Barthlott *et al.*, 1998). The cuticle has many known and suspected roles. It is the major barrier to uncontrolled water loss (Riederer and Markstädter, 1996); it controls solute loss/uptake (e.g. Mecklenburg *et al.*, 1966); is a barrier to insects and fungal pathogens; it acts as a reflective layer, so reducing leaf temperature (Cape and Percy, 1993); and it may also selectively reflect certain wavelengths of light, helping to filter out harmful uv radiation (Jagels, 1994). Any damage to the cuticle has the potential to impair its functions.

Epicuticular waxes are formed during leaf expansion, their production decelerating as leaves approach maturity (Cape, 1983). For evergreen species, wax production may continue after leaves are fully expanded. There is evidence of alterations in wax composition in older Scots pine needles (Schütt and Schuck, 1973, cited in Cape, 1983). The chemical composition of epicuticular waxes differs between species but generally the most abundant classes of compounds are lipids, aldehydes, saturated hydrocarbons and long-chain esters (reviewed in Cape, 1994).

The chemistry of epicuticular waxes of only a few individual species has been characterised. In mature *Picea pungens* needles, epicuticular wax tubules are made up of nonacosan-10-ol (Jetter *et al.*, 1996). Wax composition has been characterised throughout the ontogenetic development of the *Fagus sylvatica* leaf (Markstädter, 1994, cited in Riederer and Markstädter, 1996). As it emerges from

the bud, its waxes are dominated by long-chain alkenyl esters. In the unfolding leaf, alkan-1-ols dominate, with *n*-alkyl-*p*-coumarates and alkanes becoming abundant during the phase of maximal expansion. In mature leaves, alkanals become the dominant compound class. In *Quercus robur*, the cuticles of young leaf buds contain a homologous series of hydrocarbons, wax esters, primary alcohols, fatty acids and triterpenoids (Gulz and Muller, 1992). During May and June as leaves expand, alcohols, aldehydes and fatty acids are synthesised. In mature leaves, tetracosanol is the major component, accounting for 40% of the platelet-like wax structures (Gulz and Muller, 1992; Gulz and Boor, 1992). From July to November, wax composition remains relatively constant (Gulz and Muller, 1992).

6.1.2 Influence of environmental factors on epicuticular waxes

After wax production ceases, natural senescence leads to chemical and structural alterations in surface waxes (e.g. Sauter *et al.*, 1987; Sauter and Voss, 1986). In conifer species, tubular wax crystalloids form an intricate architecture in the somatal antechamber, and their erosion with age into flattened planar structures often leads to occlusion of the stomatal aperture (Sauter *et al.*, 1987). Environmental factors such as light, photoperiod, temperature and nutrient status can alter the morphology and chemical composition of epicuticular waxes (Cape, 1983). Air pollutants are among the environmental factors that can influence wax development and subsequent weathering. Some air pollutants retard cuticle development, possibly by interfering with biosynthetic pathways involved in wax production (Kerfourn and Gerrec, 1992). On the other hand, some pollutants have been shown to accelerate structural degradation of the cuticle, probably through direct physico-chemical reactions with surface components (e.g. Blingy *et al.*, 1973, cited in Turunen and Huttunen, 1990).

6.1.3 Studies of epicuticular waxes

Pollution-induced changes in wax structure are well documented through studies using scanning electron microscopy (reviewed in Cape, 1983). Degraded waxes

appear "melted" and lacking in fine structure. Studies of shifts in the chemical composition of the cuticle have also given insights into alterations of cuticular waxes brought about by pollution damage (reviewed in Schreuder *et al.*, 2001). A simple, indirect means of assessing damage to leaf surfaces is the study of surface wettability by measuring droplet contact angles (Leyton and Juniper, 1963). A small water droplet is placed onto the leaf surface and the angle of contact measured at the point where the droplet is advancing over the surface (Cape, 1983). Surfaces having contact angle values below 90° are "wettable", tending to favor the spreading of droplets into water films (Cape, 1983). Droplet contact angle depends on, and can therefore reflect, the chemical composition, hydrophobic/hydrophilic nature, and roughness of the surface (Cape, 1983). For instance, waxes composed of alkanes are highly hydrophobic and therefore not wettable (Turunen and Huttunen, 1990). A surface covered by tubular wax crystals does not encourage spreading of droplets, and so is less wettable than a smooth surface (Juniper, 1960; Silva-Fernandes, 1963).

6.1.4 Leaf surface wettability

As well as giving insights into physical and chemical changes in surface waxes, leaf wettability can itself have implications for the plant. More wettable surfaces are expected have a higher incidence of water film formation (Schreuder *et al.*, 2001), whilst hydrophobic surfaces tend to favor droplet formation. Free moisture on the leaf surface is essential for spore germination and subsequent hyphal growth in many fungal diseases (Huttunen, 1984). More wettable surfaces may be prone to increased pollution deposition (Grantz *et al.*, 1997), and the presence of water might accelerate reactions of gaseous pollutants with waxes (Percy and Baker, 1990). Neinhuis and Barthlott (1998) found that leaf wettability influences the retention of particulates, since particles are more easily washed off water-repellent leaves by rain.

6.1.5 Effects of air pollutants

6.1.5.1 Ozone

In fumigation studies, O_3 has been shown through SEM observations to accelerate structural degradation of epicuticular waxes of conifers and deciduous trees (reviewed in Schreuder *et al.*, 2001). Changes in wax chemistry have accompanied these ozone-induced changes in ultrastructure (e.g. Trimble *et al.*, 1982; Günthardt-Goerg and Keller, 1986). O_3 has also been associated in some studies with increased wettability of conifer needles, reflected in decreased droplet contact angles (e.g. Barnes and Brown, 1990). However, in some studies, this effect was not found even at concentrations well above the UN/ECE (United Nations Economic Commission for Europe) critical level (Cape *et al*, 1990, cited in Cape, 1994). Acid mist has been found to cause similar structural degradation (e.g. Magel and Ziegler, 1986, cited in Sauter *et al.*, 1987), but when applied in combination with O_3, no interactive effects were found (Barnes *et al.*, 1990). Maňkovská *et al.* (1998) found severe erosion of leaf surface waxes of trembling aspen grown at sites with high background levels of O_3 (which also had higher concentrations of CO_2 and particulates) compared with clean air sites. The level of damage varied between clones, in a pattern that reflected their O_3-tolerence. Different O_3 regimes have not been found to cause differential levels of damage to epicuticular waxes of *Pinus strobus* (Trimble *et al.*, 1982) and spruce (Günthardt-Goerg, 1988), so that no dose-response is evident.

O_3 is known to affect metabolic processes upon entering the plant, and so could have the potential to interfere with wax biosynthetic pathways (Cape, 1994). It may also react directly with the double bonds of unsaturated fatty acids (Heath, 1980; Sauter and Voss, 1986) or saturated hydrocarbons (Kersteins and Lendzian, 1989), which are found in the waxes of conifer needles. O_3 is associated with the generation of free radicals such as OH, which are highly reactive and are likely to interact directly with wax components (Cape, 1994). With respect to acid mist, acidic ions in solution are unlikely to cause direct

damage to cuticular components, and are thought to exert their effects indirectly (Cape, 1994).

6.1.5.2 Urban pollution

The effects on leaf surface characteristics of vehicle emissions, or individual components thereof, have been studied in the field and in controlled fumigations. In Norway spruce plants exposed at the side of a motorway for 20 weeks, Sauter *et al.* (1987) found more stomata in an advanced stage of structural degradation compared with control plants kept 15 km away. Urban *Acer platanoides* trees have been shown to sustain greater damage to their surface waxes compared with rural trees (Huttunen and Ruonala, 1986 cited in Huttunen, 1994). In a study of eight species of native Indian deciduous trees, leaf surface waxes of trees growing adjacent to roads with high traffic densities exhibited more advanced erosion compared with those at less polluted sites (Pal *et al.*, 2002). In sites showing a range of SO_2, NO, NO_2, O_3 and particulate pollution backgrounds, Cape (1983) found that older (but not current-year) needles of Scots pine became progressively more wettable in more polluted areas, indicating that sensitivity may vary with leaf age. A transect away from a nitrogen fertilizer plant gave a gradient of NO_2 and NH_3 pollution ranging from 1.8 – 8.8 μg m^{-3} and 1.8 – 45.2 μg m^{-3}, respectively. The area covered by structural wax in one-year old *Pinus sylvestris* needles increased with distance from the fertilizer plant (Kupčinskienė, 2001).

Sauter *et al.* (1987) fumigated Norway spruce with motor emissions at 560 ppb NO_x, 0.3% CO and 250 ppm hydrocarbons for 15 minutes. Even with such a short exposure period, a significantly higher percentage of stomata showed more advanced structural degradation compared with clean-air controls. In 10-day and 19-day fumigations with exhaust gasses at concentrations more typical of urban situations (100 ppb and 200 ppb NO_x), Viskari *et al.* (2000b) demonstrated acceleration of epicuticular wax degradation, also using Norway spruce. This effect was more marked in older needles than in current-year needles, and (unlike

ozone) showed a dose-response, with higher pollutant concentrations eliciting greater damage. Norway spruce fumigated with the aromatic hydrocarbons xylene and benzene, typical components of vehicle exhaust, displayed similarly degraded epicuticular waxes (Sauter and Pambor, 1989). These authors suggested that these lipophilic hydrocarbons could react directly with the cuticle, bringing about structural damage. Cape *et al.* (2003b), however, found no alteration in droplet contact angles in six British herbaceous species fumigated with a mixture of six VOCs. It should be noted that the VOCs used were typical of those found in the vicinity of a chemical installation, and only one VOC species present in the exposure (toluene) is found in appreciable amounts in vehicle exhaust.

Like some aromatic hydrocarbons, NO_x are also lipid-soluble, and have the potential to react directly with the cuticle, but this seems only to occur following long exposure to very high concentrations (e.g. Lendzian and Kersteins , 1988, cited in Cape, 1994). Jetter *et al.* (1996) found that only at very high levels of exposure (starting at 1%) did NO_2 alter the structure and chemistry of wax tubules that had been re-crystallized *in vitro* from *Picea pungens* needle surfaces. The NO_2 reacted with the wax tubules (composed of nonacosan-10-ol), transforming them into an amorphous layer made up of the final oxidation products of the original secondary alkanols. At concentrations commonly found in the atmosphere, NO_x are unlikely to be important in causing direct damage to plant cuticles (Cape, 1994), but may have the potential to cause indirect effects by disrupting biosynthetic pathways involved in wax formation.

Particulates are an important component of exhaust gas emissions. *Pinus halepensis* needles exposed to cement factory dust suffered wax damage, with crystalline waxes in the substomatal cavity coalescing into amorphous forms (Bačić *et al*, 1999). Fine particles tend to be deposited in epistomatal areas (Burkhardt *et al.*, 1995), possibly contributing to wax degradation (e.g. Grill and Golop, 1983). The mode of action by which particles could inflict damage to

cuticles is not known, but may be through direct abrasive action by mechanical impact with the surface (Cape, 1994).

6.1.6 Aims of this study

A wide range of tree and shrub species were surveyed for alterations in leaf surface wettability in response to exhaust gas exposure in the Solardomes in 2000 (Chapter 3). Two species (*Quercus robur* and *Ligustrum ovalifolium*) out of 12 showed significant, contrasting changes in droplet contact angles in response to the pollution mixtures. In the present study, droplet contact angles were measured for several of the same species as in 2000, taking measurements from different leaf age groups. This allowed a comparison between pollution-induced alterations in wettability and alterations brought about by the natural aging process. In *Quercus robur*, which showed marked alterations in leaf wettability, pollution-induced erosion of epicuticular waxes was assessed by SEM observations. To test whether pollution damage to cuticles also occurs in real urban situations, a survey was carried out of *Quercus robur* trees in areas of contrasting pollution. The degree of structural degradation of their surface waxes was compared using SEM observations.

6.2 Materials and methods

6.2.1 Plant material

Plants remaining in the Solardomes from the 2000 experimental season were used (described in section 3.2.1).

6.2.2 Droplet contact angle measurements

On 24th June 2001, when the plants had been exposed in the Solardomes for 12 months, leaves representing a range of different age classes (based on their position from the growing apex of the branch) were excised from each plant. These were immediately taken to the laboratory for droplet contact angle measurements (described in Section 2.2.5). Since the plants were under their

second year of exposure, it might be expected that the pollution would have a more marked influence on droplet contact angles. Current-year needles in conifers have been found to be less sensitive to pollution-induced alterations in surface wettability compared with older needles (Cape, 1983; Kupčinskienė, 2001).

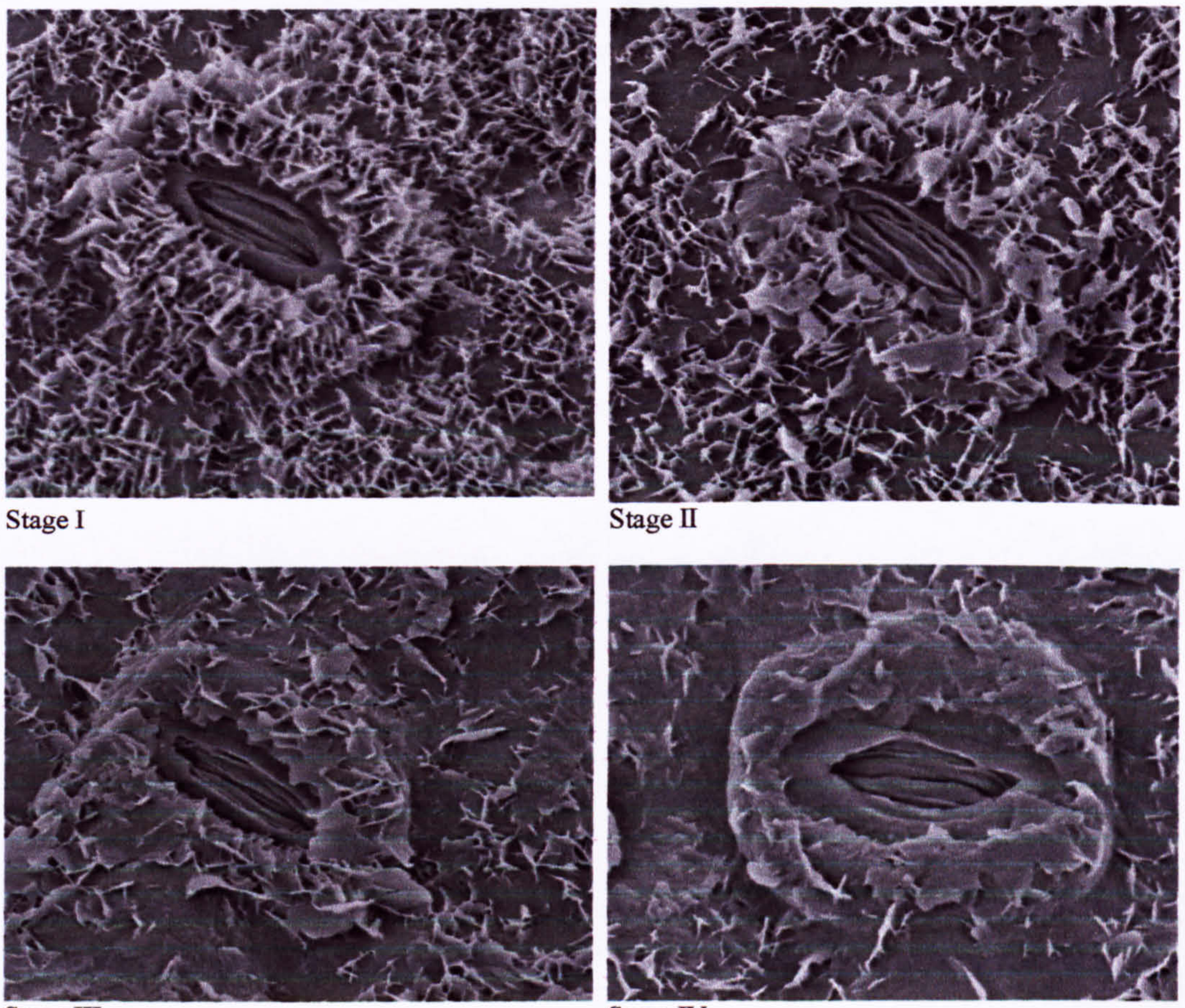
6.2.3 SEM observations

Leaves for SEM analysis were collected from *Quercus robur* saplings on 2nd October 2002, when the plants had been exposed in the Solardomes for 28 months, and current-year leaves had been exposed for the entire growing season so far of 7 months. A mature leaf was taken from each of four plants per Solardome.

For the field survey, three sites of contrasting pollution backgrounds were chosen. These were: Close House (Heddon on the Wall, Northumberland), Leazes Park and Percy Street (Newcastle Centre). These represented sites of low, medium and high levels of urban pollution, respectively. The NO_2 concentrations at each site had been monitored over 11 months (November 2001 – September 2002) using NO_2 diffusion tubes (Section 2.2.10). Four mature leaves were collected from each of three trees at each site.

Leaves were placed in a desiccator for two days to allow slow air-drying. Small sections of leaf were mounted on double-sided Sellotape on aluminum stubs, adaxial surfaces uppermost. The samples were then subjected to critical point drying and coated with gold to a thickness of 10-15 nm in a Polaron E5100 Series 2 cool sputter coater. When viewed using the SEM, the area around the stomata was found to be rich in complex crystalline wax structures, as has been found in other studies (e.g. Maňkovská *et al.*, 1998 using trembling aspen). Ten stomata per leaf were randomly selected for viewing under the SEM (Cambridge Stereoscan™ 240, Cambridge, UK) at 2000x magnification (accelerating voltage 10 kv; working distance 21 mm), and assessed for degree of structural

degradation. Stomata were assigned to one of four stages (adapted from Sauter *et al.*, 1987) of wax degradation (Table 6.1). Electron Micrographs of representative stomata depicting each stage are shown in Figure 6.1.



Stage I

Stage II

Stage III

Stage IV

Figure 6.1 Epistomatal crystalline waxes of *Quercus robur* (abaxial leaf surface) representing each of the four stages of wax degradation. Magnification x 2,000.

Table 6.1. Stages of structural degradation of waxes surrounding stomata of *Quercus robur* leaves

Stage of wax degradation	Visible condition of wax
Stage I	Pristine crystalline structure, showing no wax erosion
Stage II	The beginnings of wax erosion, with less fine detail in crystalline structures
Stage III	Advanced erosion, but with some crystalline structures still visible
Stage IV	Extremely advanced erosion, where all wax crystalline structures appear "melted"

6.2.4 Statistical Analysis

Statistics were performed using a standard SPSS statistics package (SPSS Inc., Chicago, USA). For droplet contact angles, data were checked for normal distribution and homogeneity of variance, then tested for chamber effects within treatments using ANOVA with Duncan's multiple range test. Since none were found, data from the different replicate chambers were pooled together. Twoway ANOVAs of pollution*leaf age class were used to detect overall effects of pollution. Oneway ANOVAs were performed within each age class. For numbers of stomata exhibiting stages of structural degradation, data from different treatments/field sites were compared using the χ^2 statistic.

6.3 Results

6.3.1 Droplet contact angle measurements

The different species varied in their leaf wettability, the glabrous leaves of *Cornus sanguinea* having the most hydrophobic, least wettable surfaces. Droplet contact angles generally decreased, i.e. leaves became more hydrophilic, with increasing leaf age in most of the species studied (with the exception of *Hydrangea macrophylla* "Lacecap") (Figures 6.3 – 6.7). This is as expected, since erosion of waxes is often accompanied by increased wettability.

Table 6.2 gives the main effects of exhaust gas pollution and leaf age class on droplet contact angle measurements. Only in *Quercus robur* and *Ligustrum ovalifolium* did pollution have an overall effect on leaf hydrophobicity. In *Quercus*, there was a decrease in droplet contact angle under polluted conditions (twoway ANOVA; $p<0.001$), while in *Ligustrum* pollution brought about a significant increase in contact angles (twoway ANOVA; $p=0.001$).

Hydrangea macrophylla "Pink" exhibited differences in contact angles between pollution treatments a in particular leaf age class. Only the youngest leaf age class showed a difference between treatments, with leaves from exhaust gas-polluted plants having greater droplet contact angles (i.e. more hydrophobic surfaces) compared with those from CFA (Figure 6.6). In *Ligustrum ovalifolium*, the alteration in contact angles under exhaust gas pollution was most pronounced in leaf age classes 2 and 3 (Figure 6.7).

In *Quercus robur*, there was an overall effect of pollution and of leaf age on decreasing the contact angles of water droplets with the leaf surface, but no interactive effect. Leaves showed no difference in droplet contact angles in the youngest age class of leaves. In older leaves, those from exhaust gas-polluted plants became more wettable compared with clean-air controls, with significant differences in contact angles in leaf age classes 2 and 5 (Figure 6.3).

Table 6.2 Effects of exhaust gas pollution and leaf age on contact angles of water droplets on leaves of plants grown in CFA and exhaust gas pollution. Values represent mean ±SE (n=10). Main and interactive effects of exhaust gas pollution and leaf age were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Contact angle										Main effects					
	CFA (leaf 1)	CFA (leaf 2)	CFA (leaf 3)	CFA (leaf 4)	CFA (leaf 5)	Exhaust gas (leaf 1)	Exhaust gas (leaf 2)	Exhaust gas (leaf 3)	Exhaust gas (leaf 4)	Exhaust gas (leaf 5)	Leaf age		Exhaust gas		Exhaust gas * Leaf age	
											F	P	F	P	F	P
Quercus robur	105.50 ±5.19	107.00 ±4.73	98.00 ±4.10	103.00 ±6.02	102.00 ±4.78	98.00 ±6.76	89.00 ±4.14	83.00 ±7.23	83.50 ±7.96	81.00 ±5.76	1.34	n.s.	19.45	<0.001	0.42	n.s.
Cornus sanguinea	108.50 ±7.68	99.50 ±6.64	95.00 ±5.22	90.50 ±9.67	———	104.50 ±3.69	96.50 ±4.35	93.50 ±5.53	98.50 ±4.89	———	1.684	n.s.	0.01	n.s.	0.39	n.s.
Hydrangea "Lacecap"	63.50 ±7.23	66.50 ±6.46	75.00 ±6.75	———	———	80.00 ±4.08	63.50 ±6.91	77.50 ±4.67	———	———	1.70	n.s.	1.13	n.s.	1.34	n.s.
Hydrangea "Pink"	70.00 ±4.08	68.50 ±6.06	71.00 ±4.82	———	———	84.50 ±4.44	69.50 ±6.60	65.50 ±4.04	———	———	1.91	n.s.	0.64	n.s.	1.99	n.s.
Ligustrum ovalifolium	94.50 ±2.83	87.50 ±4.49	87.00 ±3.67	87.00 ±3.82	84.00 ±4.14	101.00 ±2.67	98.00 ±2.60	99.00 ±3.48	91.50 ±4.41	90.00 ±3.94	2.50	0.048	11.62	0.001	0.38	n.s.

Leaves of age classes 3 and 4 also had lower contact angles in polluted compared with clean air conditions, although not significantly ($p = 0.088$ for age class 3; $p = 0.066$ for age class 4). If waxes were being eroded by the exhaust gas pollution, it would be expected that older leaves, which had been exposed for a longer time, should become progressively more wettable. In age class 2, mean

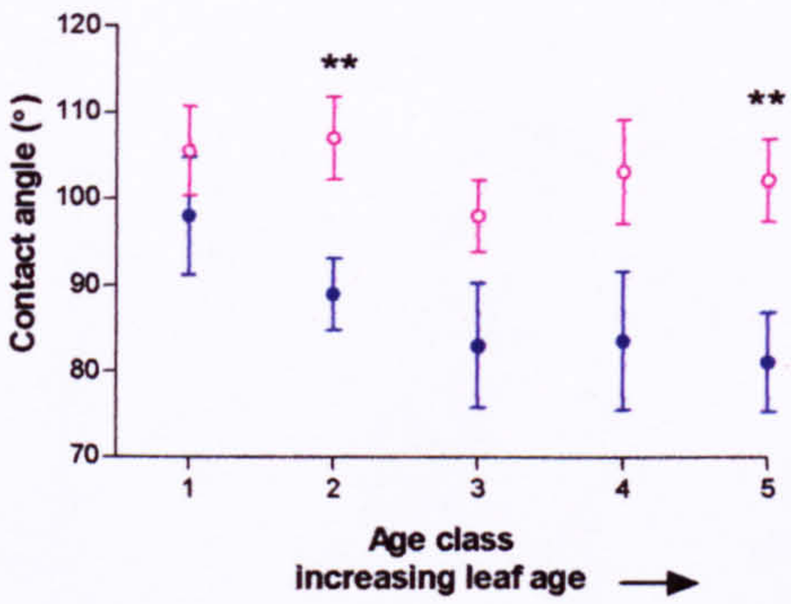


Figure 6.3 Angles of contact of water droplets (mean ± SE) on *Quercus robur* leaves of different ages in CFA (○) and exhaust gas-polluted air (●; 100 ppb NO_x). Oneway ANOVA were performed within each age class. Asterisks denote the probability of difference between CF and polluted air (** $p<0.01$). n=10.

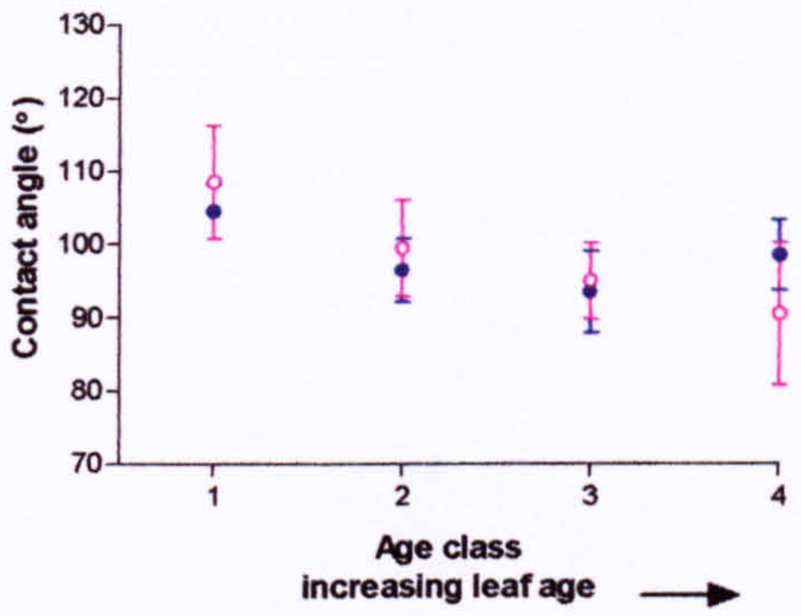


Figure 6.4 Angles of contact of water droplets (mean ± SE) on *Cornus sanguinea* leaves of different ages in CFA (○) and exhaust gas-polluted air (●; 100 ppb NO_x). n=10.

Figure 6.5 Angles of contact of water droplets (mean ± SE) on *Hydrangea macrophylla* "Lacecap" leaves of different ages in CFA (○) and exhaust gas-polluted air (●; 100 ppb NO_x). n=10.

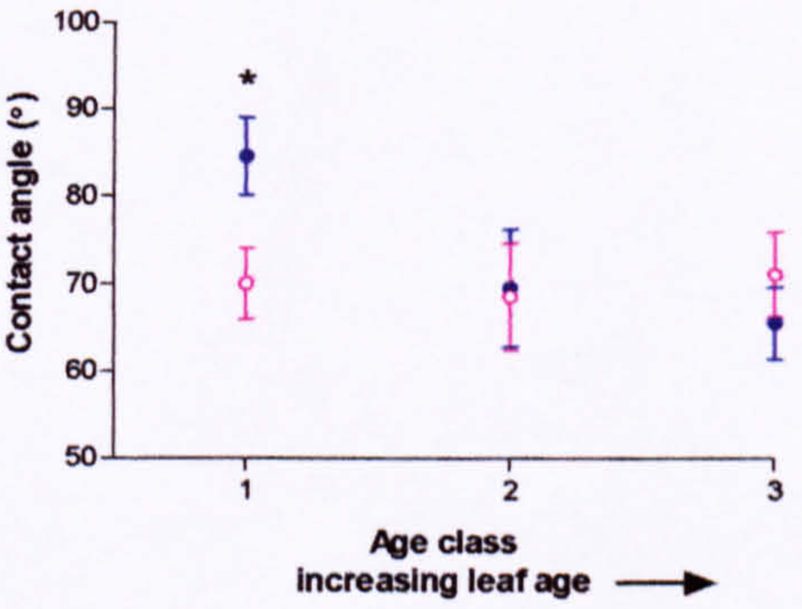


Figure 6.6 Angles of contact of water droplets (mean ± SE) on *Hydrangea macrophylla* "Pink" leaves of different ages in CFA (○) and exhaust gas-polluted air (●; 100 ppb NO_x). Asterisks denote the probability of difference between CF and polluted air (* $p<0.05$). n=10.

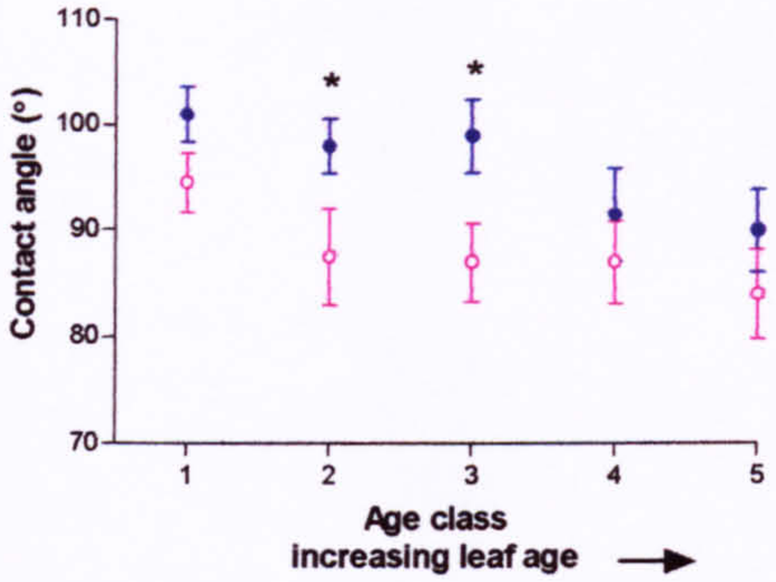


Figure 6.7 Angles of contact of water droplets (mean ± SE) on *Ligustrum ovalifolium* leaves of different ages in CFA (○) and exhaust gas-polluted air (●; 100 ppb NO_x). Asterisks denote the probability of difference between CF and polluted air (** $p<0.01$). n=10.

droplet contact angles of leaves in clean air were 107° compared with 89° for leaves from exhaust gas-polluted plants. For age class 5, mean angles of contact were 102° and 81° for clean air and polluted leaves, respectively. The mean contact angle values of leaves from clean air were above the 90° threshold of droplet as opposed to water film formation. Leaves from polluted conditions had mean contact angles below 90°, so that water films, rather than droplets, are more likely to be formed.

6.3.2 SEM observations on *Quercus robur* plants from the Solardome experiment

The abaxial leaf surface of *Quercus robur* leaves is covered by a dense arrangement of fringe-edged platelet-like epicuticular wax structures, concentrated around the stomatal rim. Four stages of wax degradation ranging from pristine platelets to an advanced state of erosion (Table 6.1, Figure 6.1) were identified and used to score stomata.

The percentage frequencies of stomata exhibiting each stage of wax degradation from *Quercus* saplings grown in clean air and exhaust gas-polluted air in the Solardomes are given in Figure 6.8. Scores were pooled and then compared

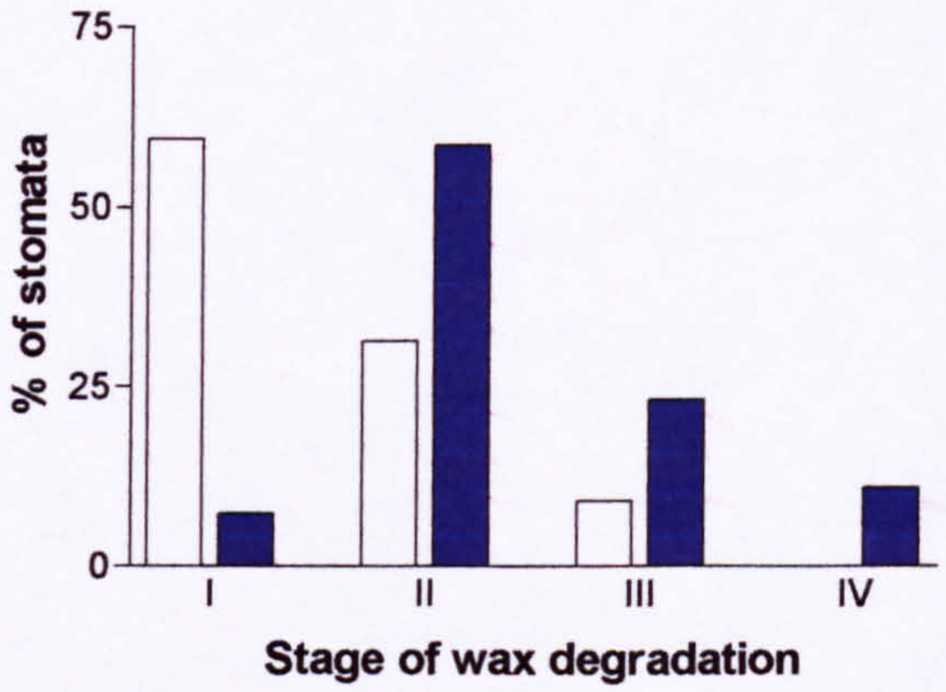


Figure 6.8 Percentage frequency of stomata in each stage of wax degradation from *Quercus robur* leaves in CFA (□) and exhaust gas-polluted air (▼; 100 ppb NO_x). A χ^2 test showed significant ($p<0.001$) differences between treatments.

Table 6.3 Comparison of stages of wax degradation in *Quercus robur* leaves from clean-air and exhaust gas-polluted air Solardomes. Comparison of treatments is by χ^2 with 3 d.f. Expected values are given in brackets. $\chi^2 = 62.97$; $p<0.001$ **

Wax stage	**Clean air**	**Exhaust gas-polluted air**	Total
I	**72 (46.49)**	**6 (31.51)**	78
II	**38 (51.26)**	**48 (34.74)**	86
III	**11 (17.88)**	**19 (12.12)**	30
IV	**0 (5.36)**	**9 (3.64)**	9
Total	121	82	203

using the χ^2 statistic (Table 6.3). This showed a significant difference ($p<0.001$) between treatments. Waxes from exhaust gas-polluted plants appeared to be shifted towards greater stages of erosion compared with clean-air controls. The commonest state for stomata from control plants was Stage I (59.5% of stomata), while for exhaust gas-polluted plants this was Stage II (58.5% of stomata). No

control plants displayed any stomata in the most advanced stage of structural degradation (Stage IV), while 10.9% of exhaust-gas polluted plants did.

6.3.3 Field survey of *Quercus robur* trees

6.3.3.1 Pollution monitoring at field sites around Newcastle upon Tyne

Close House had the lowest average NO_2 concentration at 8.7 ppb, Leazes Park was intermediate at 22.1 ppb, and Percy Street was the highest at 45.7 ppb (Figure 6.9).

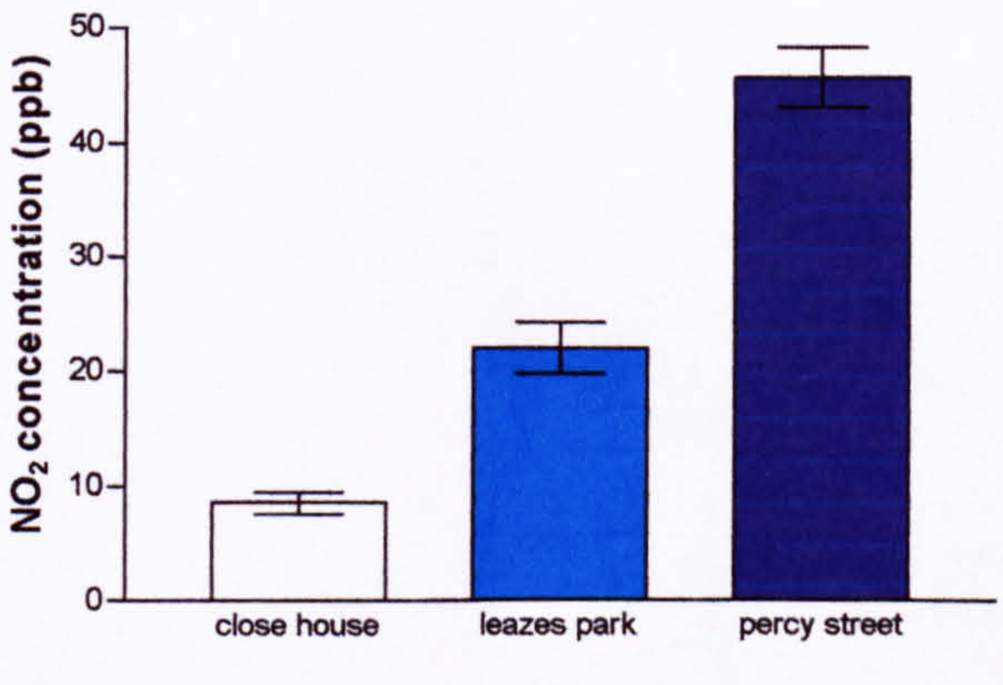


Figure 6.9 Monthly NO_2 concentrations (mean ± SE) for November 2001 – September 2002 at sites around Newcastle upon Tyne of low (□), moderate (▬) and high (▬) urban pollution.

6.3.3.2 SEM observations

Figure 6.10 shows the percentage frequencies of stomata exhibiting each stage of wax degradation from mature *Quercus* trees from three sites of contrasting urban pollution background concentrations. Pooling of scores and comparison using the χ^2 statistic (Table 6.4) gave significant differences ($p<0.001$) in wax degradation between sites. The cleanest site had 61.7% of stomata in stage I, compared with 31.4% and 14.8% at the moderately and heavily polluted sites, respectively (Figure 6.10). The three sites had similar percentages of stomata exhibiting stage II stomata. The percentage of stomata in more advanced stages of structural

degradation (Stages III and IV combined) was 6.5% for trees from the clean air site, compared with 38.1% at the moderately polluted site, and 55.7% at the heavily polluted site.

These results indicate clear evidence of urban pollution-induced structural degradation of epicuticular waxes in *Quercus robur*.

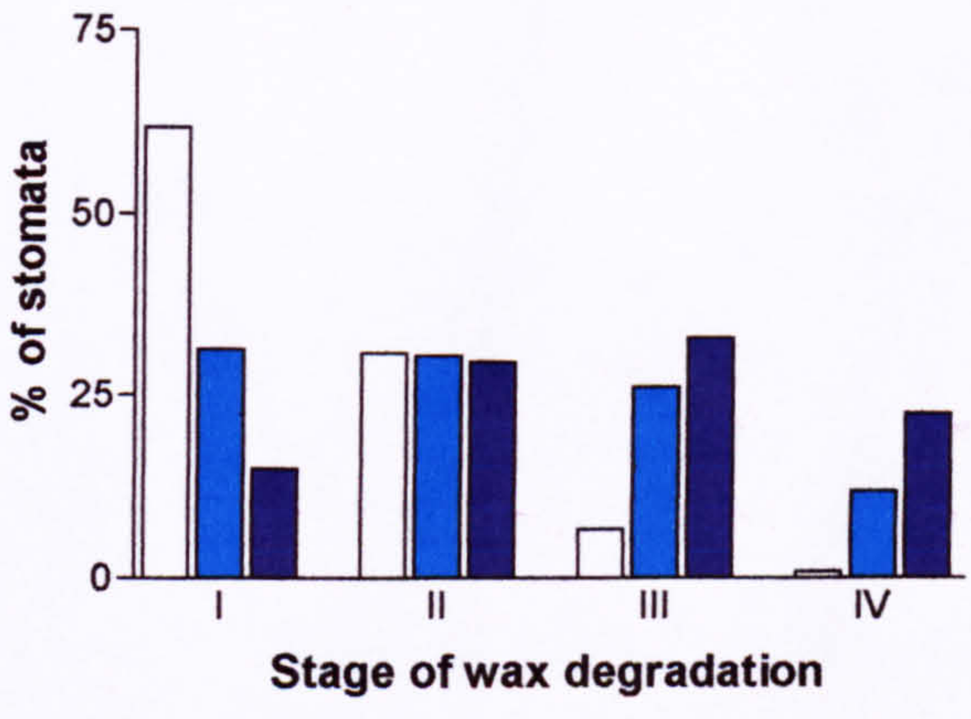


Figure 6.10 Percentage frequency of stomata in each stage of wax degradation from *Quercus robur* leaves from sites with low (□), moderate (▬) and high (▬) levels of NO_2 pollution. A χ^2 test showed significant ($p<0.001$) differences between sites.

Table 6.4 Comparison of stages of wax degradation in *Quercus robur* leaves from sites of contrasting urban pollution backgrounds. Comparison of treatments is by χ^2 with 6 d.f. Expected values are given in brackets. $\chi^2 = 81.14$; $p<0.001$ **

Wax stage	**Close House (8.7 ppb average NO_2)**	**Leazes Park (22.1 ppb average NO_2)**	**Percy Street (45.7 ppb average NO_2)**	Total
I	**74 (43.51)**	**37 (42.79)**	**17 (41.70)**	128
II	**37 (36.37)**	**36 (35.77)**	**34 (34.86)**	107
III	**8 (26.18)**	**31 (25.74)**	**38 (25.08)**	77
IV	**1 (13.94)**	**14 (13.71)**	**26 (13.36)**	41
Total	120	118	115	353

6.4 Discussion

Several types of pollution have been found to cause damage to leaf surface waxes in conifers and several broadleaved species. This has been reflected in alterations in the water-repellency, chemical composition and wax structure of leaf cuticles.

In *Hydrangea macrophylla* "Pink" and *Ligustrum ovalifolium*, leaves of some age classes had greater droplet contact angles in exhaust gas-polluted air compared with clean-air controls. This situation is opposite to what would be expected. Since contact angles tended to decrease with age as the leaves underwent natural weathering, the pollution-induced increase in contact angle is unlikely to reflect an accelerated aging effect, which should mimic acceleraed natural senescence. Perhaps the chemical composition of the surface waxes in these species is such that interaction with pollutants caused increased water-repellency. It is also possible that contaminants from the pollution mixture deposited on the leaves had hydrophobic properties. The micromorphology of the leaf surface might also influence the response to air pollutants. When *Ligustrum* leaves were viewed using the SEM, their surfaces were smooth and featureless, lacking complex epicuticular wax crystals. It is not known how this might affect the response to pollutant deposition.

The condition of surface waxes of *Quercus robur* leaves proved to be a good indicator for urban pollution damage. In saplings in the Solardome chambers, wax degradation with age was reflected in a progressive decrease in droplet contact angle values, so that leaves became gradually less water-repellent as they aged. The rate of this decline appeared to be accelerated in leaves of plants from exhaust gas-polluted air compared with clean air controls (Figure 6.3). This age effect demonstrates the importance of using leaves of similar ages when making comparisons of droplet contact angles.

The changes in leaf wettability in exhaust gas-polluted *Quercus robur* saplings were probably the result of the observed alterations in the crystal habits of their epicuticular wax structures. This may have been accompanied by alterations in chemical composition of the surface waxes, although this was not assessed in the present study. In observations using the SEM, the platelet-like wax crystals surrounding stomata were significantly more eroded in leaves from exhaust gas-polluted plants compared with clean air controls. Leaves grown in exhaust gas-polluted air were shifted towards the more advanced stages of structural degradation compared with those that developed in clean air (Figure 6.8, Table 6.3).

It has been suggested that, since many environmental factors can influence wax morphology, microclimatic effects may override and mask effects of air pollutants (Gunthardt-Goerg, 1988). Factors such as temperature, light, wind and rain can affect the rate of production as well as the morphology and chemistry of waxes (Turunen and Huttunen, 1990). In order to address this question, a survey was performed on mature *Quercus robur* trees at sites with different pollution concentrations. The survey showed that the effect found under the controlled environmental conditions of the Solaredome chambers also occurred in real urban situations. Wax crystalloids on leaves from trees in more heavily polluted sites were again clearly shifted towards more advanced stages of erosion compared with those from a clean site (Figure 10, Table 6.4). The damage to surface waxes was greatest at the site with highest pollution concentrations, so that there appeared to be a dose effect, although to prove this conclusively, more sites representing a greater range of pollution backgrounds would need to be sampled.

The observed alterations in the surface structure and water-repellency of *Quercus robur* leaves could have consequences for pollution deposition and damage to the cuticle. Increases in wettability of the leaf surfaces induced by the urban pollution mixtures were such that the droplet contact angle values for leaves in

polluted conditions were below the 90° threshold of wettability (Cape, 1983) in all but the youngest leaf age class. In contrast, leaves in clean air retained greater than 90° droplet contact angles, even in the oldest leaf class (Figure 6.4). The pollution therefore appeared to induce *Quercus* leaf surfaces to switch from being "non-wettable", favoring droplet formation, to being "wettable", with a tendancy to form moisture films. This might be expected to exacerbate pollution damage, since more wettable leaf surfaces are expected to attract greater deposition of gaseous pollutants (Grantz *et al.*, 1997), greater retention of particulates (Neinhuis and Barthlott, 1998), and may be more prone to chemical reactions with pollutants (Percy and Baker, 1990).

The plants in the present study, both in the Solardome glasshouses and in field surveys, were exposed to the full complement of complex urban pollution mixtures. It is therefore not possible to know which component of the vehicle emissions was responsible for the observed effects on leaf surface waxes. NO_x are a possible candidate for indirect effects on wax syntesis, but they only undergo direct reactions with surface components at much higher concentrations than were present here (Lendzian and Kersteins, 1988, cited in Cape, 1994; Jetter *et al.*, 1996). Acid mist has been shown to cause structural degradation of waxes (Magel and Ziegler, 1986, cited in Sauter *et al.*, 1987; Barnes *et al.*, 1990), probably also by indirect means. Precipitation in urban areas is acidic, and might have a similar effect. VOCs and particulates are the most likely components that interact directly with waxes, accelerating their degradation. VOCs have the ability to dissolve in and react with wax lipids (Sauter and Pambor, 1989), and have been shown to cause erosion of waxes similar to that observed in urban pollution climates (Sauter *et al.*, 1987). Particulates are suspected to physically impact with leaf surfaces, with the potential to cause abrasive damage (Cape, 1994).

Chapter 7: The Effects of Urban Pollution on Infection by Tar Spot Disease

7.1 Introduction

7.1.1 Life cycle of *Rhytisma acerinium*

Tar spot disease of sycamore (*Acer pseudoplatanus*) is caused by the Ascomycete fungus *Rhytisma acerinum* (Pers.) Fries. The fungus overwinters in the form of black stromata on sycamore leaf debris. In late spring (usually during May), under suitable environmental conditions, the stromatal layers become swollen through the absorption of water and are pushed back to expose the apothecia. The subsequent release of the gelatinous sheathed ascospores is triggered by alterations in humidity (Greenhalgh and Bevan, 1978). The ascospores are ejected from the surface of the apothecia to a height of 1 mm (Muller, 1912, cited in Leith and Fowler, 1987), and are carried in the air by eddies away from the ground, where they may contact newly-expanded sycamore leaves. The presence of free moisture on the leaf surface is essential for successful spore germination and growth prior to infection (e.g. Huttunen, 1984). For the first 6-8 weeks, infection is asymptomatic (Muller, 1912, cited in Leith and Fowler, 1987), after which small black spots surrounded by a yellow margin appear on the adaxial surface of the leaf. These stromata increase in size to become the characteristic black "tar spots" shown in Figure 7.1.

Figure 7.1. *Rhytisma acerinium* infection on a Sycamore leaf.

7.1.2 *Rhytisma* and SO_2 pollution

There are several early observations of the apparent inhibition of *Rhytisma* on sycamore (reviewed in Bevan and Greenhalgh, 1976) and maple (reviewed in Heagle, 1973) in areas of high industrial/urban pollution. Tar spot disease was noted to be uncommon in cities since at least the 1950s. Until the 1980s, the consensus was that SO_2 was the major pollutant responsible for the lack of tar spot on urban sycamores. The incidence of several other fungal diseases is known to be affected by air pollutants (reviewed in Heagle, 1973 and Bell *et al.*, 1993). For example, blackspot infection of *Rosa* species by *Diplocarpan rosae* has been shown in field observations to be eliminated above a threshold SO_2 concentration, and in fumigations with SO_2, infection was shown to be reduced (Saunders, 1966, cited in Bell *et al.*, 1993). This SO_2 effect was not a result of its acidity. Germination rates of conidia in cultures containing various SO_2-precursors (e.g. H_2SO_3, H_2SO_4, HCl, HNO_3, H_3PO_4) showed no correlation with pH (Saunders, 1965).

Bevan and Greenhalgh's (1976) and Greenhalgh and Bevan's (1978) studies attempted to demonstrate a link between inhibition of *Rhytisma* and SO_2 pollution. In several field surveys, they showed the severity of infection to be inversely proportional to the mean annual SO_2 concentration, with complete absence of the disease above a threshold of 85-90 $\mu g\ m^{-3}$ SO_2 (Bevan and Greenhalgh, 1976). The severity of infection is measured using the tar spot index (calculated from the number of spots per 100 cm^2 of leaf). Bevan and Greenhalgh's studies indicated that it is during the infection period that *Rhytisma* is sensitive to SO_2 pollution. In saplings infected in a woodland of relatively clean air and subsequently transported to Liverpool City centre (125 $\mu g\ m^{-3}$ SO_2), the disease developed normally (Bevan and Greenhalgh, 1976). Control saplings retained in Liverpool and supplied with inoculum did not become infected by the disease (Greenhalgh and Bevan, 1978). These studies suggested that tar spot could be used as a biological indicator of air pollution.

Leith and Fowler (1987) observed that tar spot was absent from Edinburgh city centre, where background SO_2 levels were below Bevan and Greenhalgh's (1976) proposed threshold for pollutant effects on *Rhytisma*. They suggested that the lack of infection in cities could be due to factors other than air pollution, and tested the hypothesis that the removal of inoculum (by wind and human activities) was the major cause of the low incidence of tar spot in cities. In their survey of trees in both urban and rural situations, Leith and Fowler (1987) found that leaf litter was removed prior to the spring infection period at most sites in the city. At rural sites, the amount of inoculum present depended on the exposure to wind of areas surrounding individual trees, with little opportunity for the accumulation of leaf litter at exposed sites. Tar spot indices of trees taken in autumn were a function of the amount of inoculum found below the trees in the preceding spring.

Leith and Fowler's (1987) study also included an experiment where sycamore saplings were infected at three sites of varying background SO_2 concentrations. Each site also included a control group that was not supplied with inoculum. Where inoculum was present, all saplings contracted tar spot disease, irrespective of the local SO_2 concentration. Virtually no infections occurred on non-inoculated controls. However, Leith and Fowler's most polluted site had a mean daily SO_2 concentration of only 41 $\mu g\ m^{-3}$, which may have been insufficient to inhibit infection by *Rhytisma*. Leith and Fowler (1987) have suggested that SO_2 may have played a role in the initial disappearance of tar spot disease from urban areas, its re-invasion being hampered by the removal of leaf litter.

7.1.3 *Rhytisma* and current urban pollution climates

During the past decade, the typical urban pollution climate in the UK has changed markedly, with dramatic declines in SO_2 emissions. Other pollutants, most notably NO_x have increased in urban zones. Tar spot has re-invaded some urban areas, while other areas have remained clear. Recent work has pointed to a

role of NO_2 in the distribution of tar spot. Jarraud (2000) performed both field surveys and transplant experiments along a transect in London representing an air quality gradient. The natural distribution of the disease appeared to be negatively correlated with NO_2 concentration, and Jarraud gives an estimated annual average threshold concentration of 15 ppb NO_2, above which infection is negligible. His results also point to a possible positive correlation of tar spot incidence with O_3 concentration, but this relationship was found in only one of the three years of study. This could be an artefact of the relationship between concentrations of NO_2 and O_3, since NO_2 declines as O_3 increases with distance from urban centres. Jarraud's (2000) separate transplant experiments, where saplings were planted along the same transect used for the field surveys, and exposed to standard amounts of inoculum, confirmed the negative relationship between disease incidence and NO_2, but gave no correlation with O_3. Although Jarraud (2000) found a correlation between NO_2 concentrations and the incidence of tar spot disease, NO_2 is not necessarily the causal agent in suppressing the disease. NO_2 is present in urban environments in combination with a complex mixture of other vehicle-derived pollutants.

7.1.4 Aims of this study

A unique opportunity presented itself in the present study to examine the development of tar spot disease under known concentrations of exhaust gases under the controlled experimental conditions of the urban pollution Solardomes. To complement these glasshouse experiments, and to compare Jarraud's (2000) findings with a less heavily polluted city than London, a survey of tar spot disease incidence was carried out in Newcastle upon Tyne.

7.2 Materials and methods

7.2.1 The tar spot index (TSI)

The tar spot index refers to the number of tar spots per 100 cm^2 leaf area. Leaves were removed and placed in plastic bags to prevent desiccation. Leaf areas were

measured using a Delta-T area meter (Delta-T devices Ltd., Burwell, Cambridge, UK), and the number of tar spots for each leaf was recorded. Spot number for each leaf was divided by the leaf area (cm^2) and multiplied by 100 to give TSI.

7.2.2 Solardome studies

7.2.2.1. Accidental infection in 2000

In June 2000, twenty one-year old *Acer pseudoplatanus* saplings were purchased from from Cheviot Trees (Berwick upon Tweed, Northumberland). The plants were potted as described in Section 2.2.1 and five plants were assigned each Solardome glasshouse. In August 2000, the symptoms of tar spot disease were observed. Since the plants were infected before being placed in the Solardomes, the effect of the pollution treatment on the *development* only of the disease could be determined. All the leaves were removed from the saplings in September, once the tar spots were fully developed, and the tar spot indices were recorded.

7.2.2.2 Controlled inoculation in 2002

Forty one-year old *Acer pseudoplatanus* saplings were purchased in March 2001 from Cheviot Trees (Berwick upon Tweed, Northumberland) and ten plants placed in each Solardome glasshouse. An attempt was made to infect the plants with tar spot in 2001, but was unsuccessful. In March 2002, using the same plants that were purchased in 2001 (now two years old), 20 g of infected leaf litter was placed in each of four seed trays and each tray was covered with a lid of garden mesh. An equal weight of infected litter was used in each treatment in an attempt to standardise the dose of inoculum. One tray was placed in each Solardome and the sycamore saplings were positioned, equally spaced, around the tray. The infected litter was sprayed daily with water to encourage spore release. The leaves of the saplings were also sprayed daily, until the end of May, past the usual infection period. In September 2002, all the leaves were removed from the saplings and tar spot indices were recorded.

7.2.3 Field surveys

7.2.3.1 Inoculum and tar spot counts.

In 2001, the incidence of tar spot disease on sycamores in autumn was studied at eight urban sites covering a range of NO_2 concentrations. These were: Leazes Park, High Dene, Hunters Road, Westgate Road, Richardson Road, Jesmond Dene Road, Shields Road and Percy Street (in order of ascending local NO_2 concentrations). Four well-spaced trees per site were studied. Counts of inoculum were made in April, just prior to the usual infection period. This was measured as the number of overwintered tar spots present in the leaf litter in a 1 m^2 quadrat at the base of each tree (as described in Leith and Fowler, 1987), randomly positioned within a 4 m radius of the trunk. The same trees were revisited in October, and TSIs recorded for samples of 20 leaves from each tree. Leaves were collected at head height with eyes closed to ensure random sampling. The same sampling procedure was repeated in 2002, with the addition of three clean air sites in a rural area, Close House.

7.2.3.2 Measurement of NO_2 concentrations

Monthly measurements of NO_2 concentrations at each site were taken between November 2001 and September 2002 using passive NO_2 diffusion tubes as described in Section 2.2.10. For the rural sites, NO_2 data were collected for May 2002 – July 2002.

7.2.4 Statistical Analysis

Data were checked for normal distribution and homogeneity of variance. For the Solardome studies, tar spot indices of individual chambers were compared by oneway ANOVA using Duncan's multiple range test. Data of percentage of leaves bearing tar spots were compared using Duncan's multiple range test after being Arcsine transformed. For parameters measured at field sites, linear regressions were determined in order to elucidate any effects of NO_2 concentration on the presence of the disease.

7.3 Results

7.3.1 Solardome studies

7.3.1.1 Accidental infection in 2000

Mean tar spot indices for sycamore saplings in polluted and clean air Solardomes did not differ significantly, ranging from 2.34 - 2.58 (Figure 7.2). The percentages of leaves in each Solardome showing infection are given in Figure 7.3. They were not related to pollution regime. Because infection occurred before the plants were introduced into the Solardomes, these results can only show any possible influence of urban pollution mixtures on development of the disease, but not on infection rates.

7.3.1.2 Controlled inoculation in 2002

Tar spot indices for sycamore saplings infected in the Solardomes under urban pollution mixtures are shown in Figure 7.4. Mean TSIs for the clean air Solardomes were 4.55 ± 0.08 and 23.29 ± 2.59 compared with 0.10 ± 0.04 and 0.08 ± 0.06 in polluted Solardomes. The percentages of leaves showing infection were significantly higher for plants grown in clean air compared with those in polluted air (Figure 7.5).

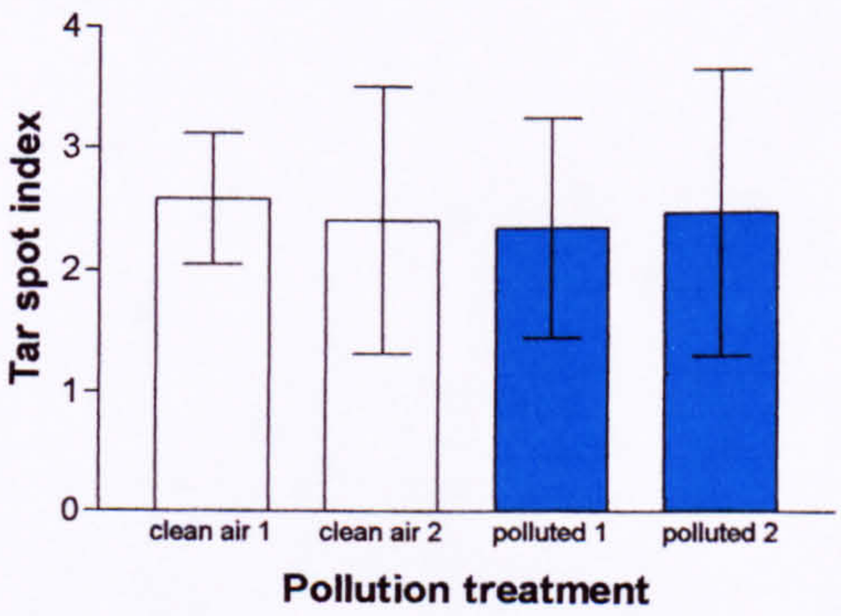


Figure 7.2 Tar spot index (mean ± SE) for saplings from the Solardomes in September 2000. CFA (□); exhaust gas-polluted air (ν; 100 ppb NO_x). Infection occurred prior to fumigation. n = 5.

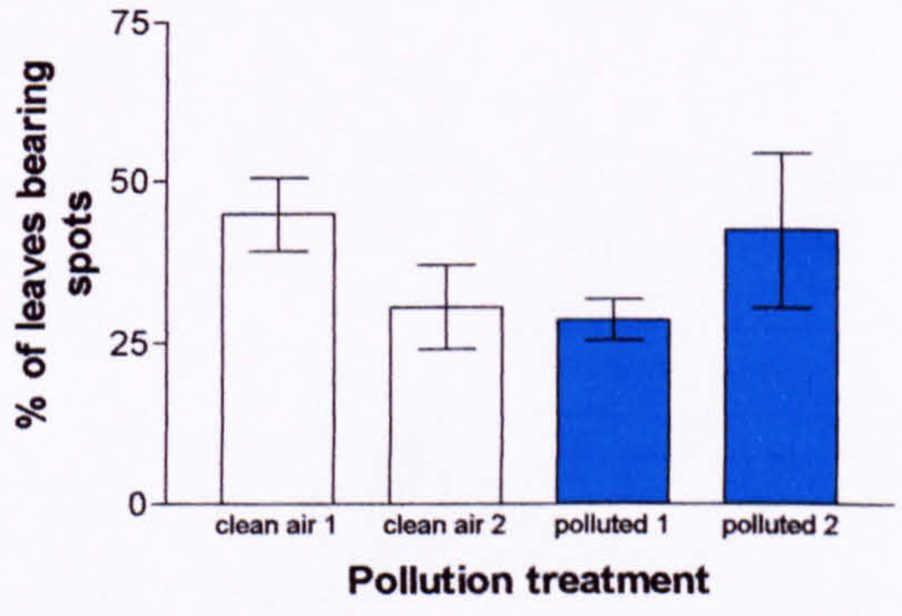


Figure 7.3 Percentage of leaves (mean ± SE) bearing tar spots in saplings from the Solardomes in September 2000. CFA (□); exhaust gas-polluted air (ν; 100 ppb NO_x). Infection occurred prior to fumigation. Data have been Arcsine transformed. n = 5.

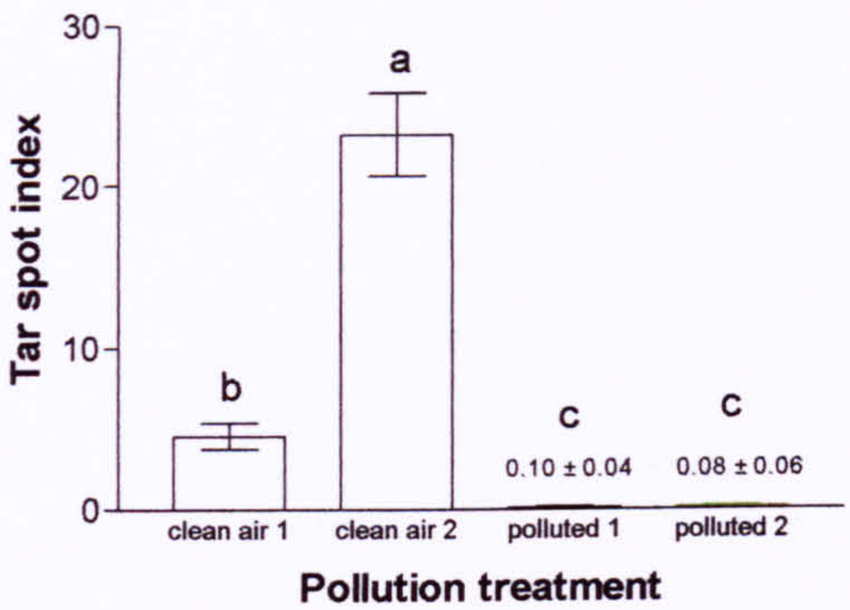


Figure 7.4 Tar spot index (mean ± SE) for saplings from the Solardomes in September 2002. CFA (□); exhaust gas-polluted air (ν; 100 ppb NO_x). Infection occurred during fumigation. n = 10. Different letters denote significant ($p<0.05$) differences between Solardomes (Duncan's multiple range test).

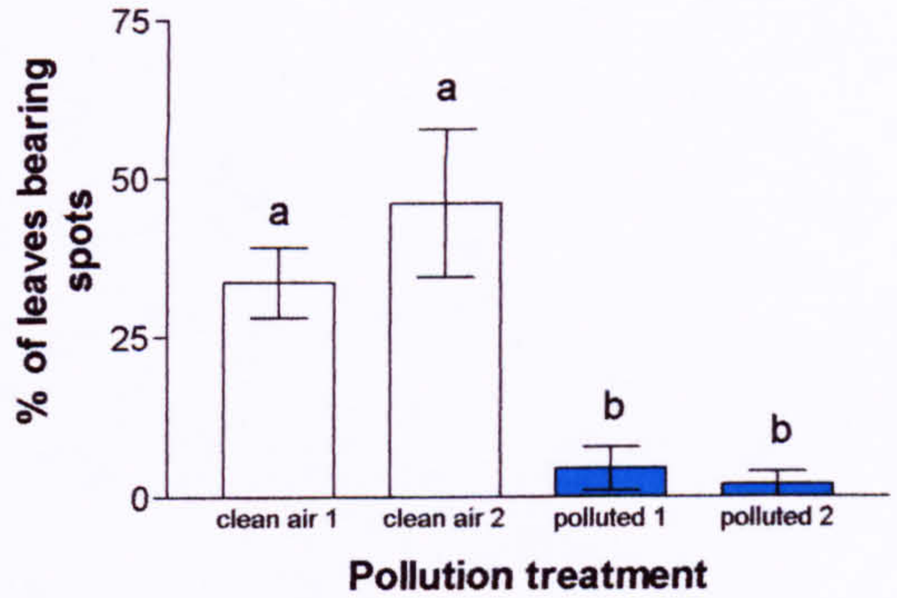


Figure 7.5 Figure 3. Percentage of leaves (mean ± SE) bearing tar spots in saplings from the Solardomes in September 2000. CFA (□); exhaust gas-polluted air (ν; 100 ppb NO_x). Infection occurred during fumigation. Data have been Arcsine transformed. n = 10. Different letters denote significant ($p<0.05$) differences between Solardomes (Duncan's multiple range test).

7.3.2 Field surveys

Figure 7.6 shows, for each study site, the average of monthly measurements of NO_2 concentrations between November 2001 and September 2002, as well as the NO_2 concentration in May 2002 (the usual tar spot infection period). Mean NO_2 concentrations ranged from 8.7 ppb to 45.7 ppb, indicating that the sites represented a good spread of background urban pollution levels.

It is unclear from the inoculum counts made in April 2001 whether there was a relationship between the number of overwintered tar spots remaining below trees and average NO_2 concentrations, possibly because there were few sites with low NO_2 levels (Figure 7.7). With the addition of three extra clean air sites to the survey made in April 2002, a relationship became apparent (Figure 7.9), with the density of inoculum being negatively correlated with average NO_2 concentration ($y = -2.2249x + 87.11$, $r^2 = 0.65$, $p = 0.0028$). TSIs taken in October 2001 and

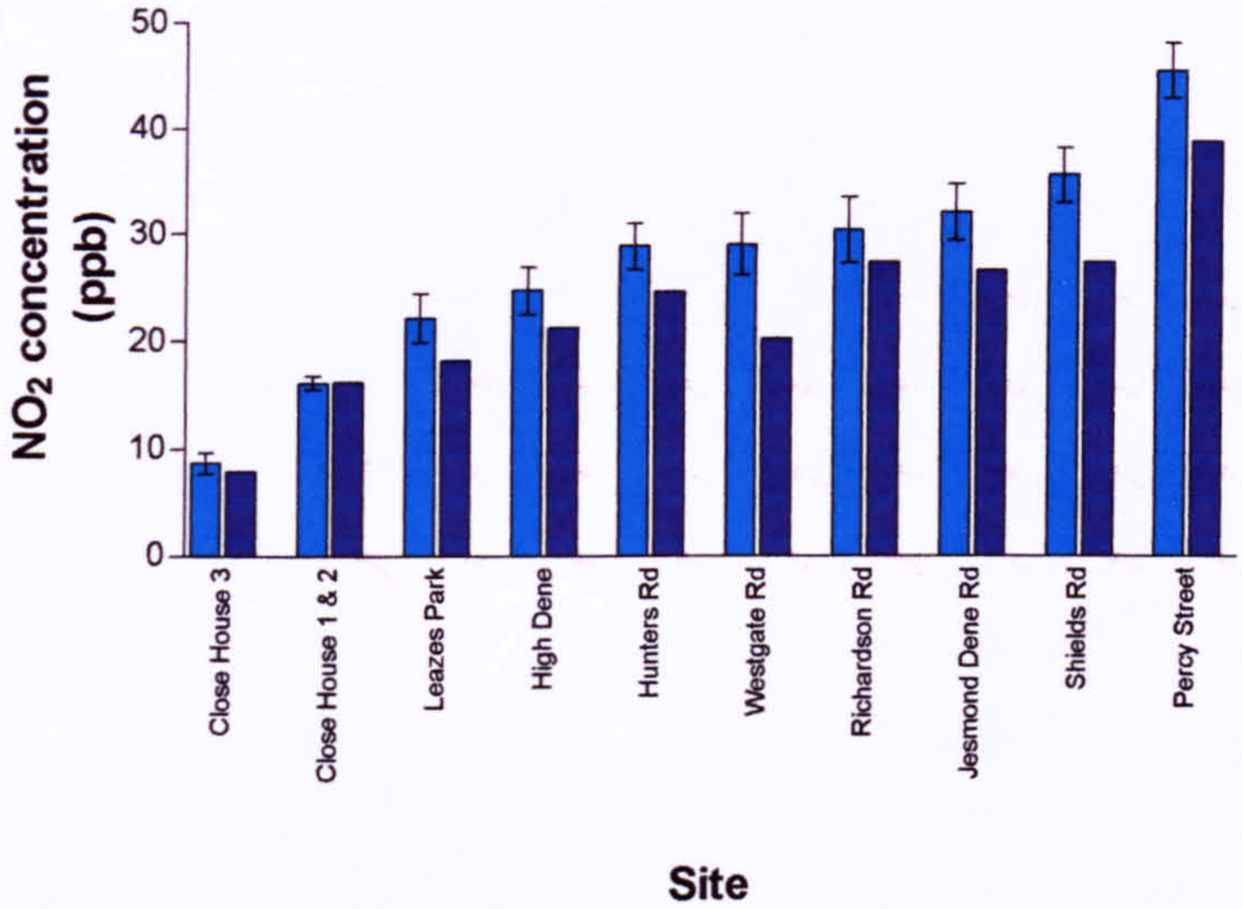


Figure 7.6 Monthly NO_2 concentrations (mean ± SE) for Nov 2001 – Sept 2002 (v) and NO_2 concentrations for May 2002 (v) at the study sites. (Data for Close House sites only from May – July 2002)

2002 are not significantly related to NO_2 concentration (Figures 7.8 and 7.10). For infections in October 2002, it was possible to plot TSIs against NO_2 concentrations in May 2002, when infection would have occurred. No significant relationship was found (Figure 7.11).

The amount of inoculum present in spring did not correlate with tar spot infection in autumn (Figure 7.12). This suggests that the quadrats of leaf litter were not representative of the inoculum available for infection of the trees. In many cases, no inoculum was detected at the base of the tree, but TSI values were high (Figure 7.12).

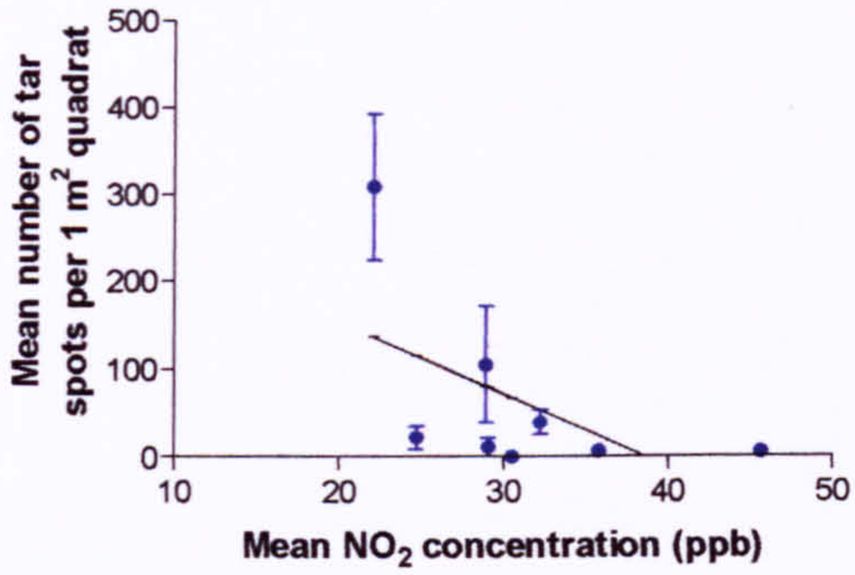


Figure 7.7 Numbers of overwintered tar spots (mean ± SE) in 1 m^2 quadrats in April 2001 plotted against mean NO_2 concentration (Nov 2001 – Sept 2002) for each site. $y = -8.3031x + 320.68$, $r^2 = 0.32$, $p = 0.14$

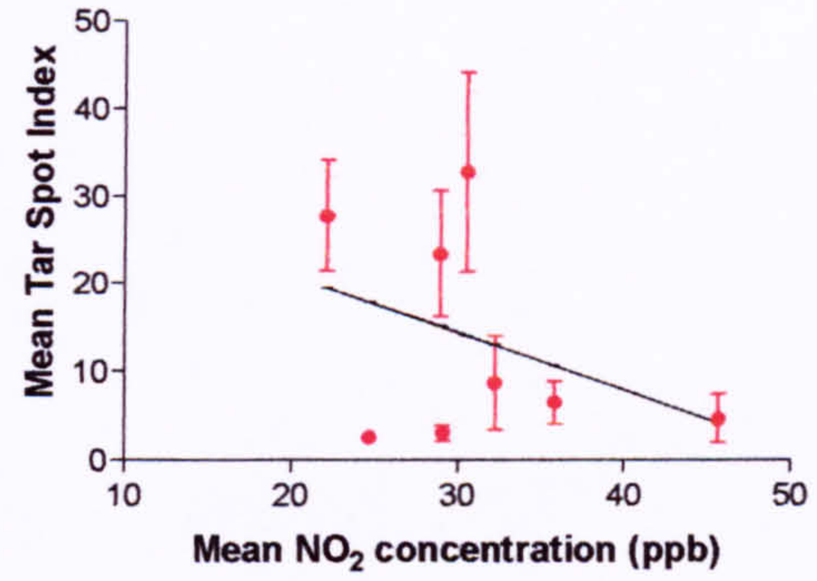


Figure 7.8 TSI for each site (mean ± SE) in Oct 2001 plotted against mean NO_2 concentration (Nov 2001 – Sept 2002) for each site. $y = -0.65x + 33.878$, $r^2 = 0.15$, $p = 0.35$

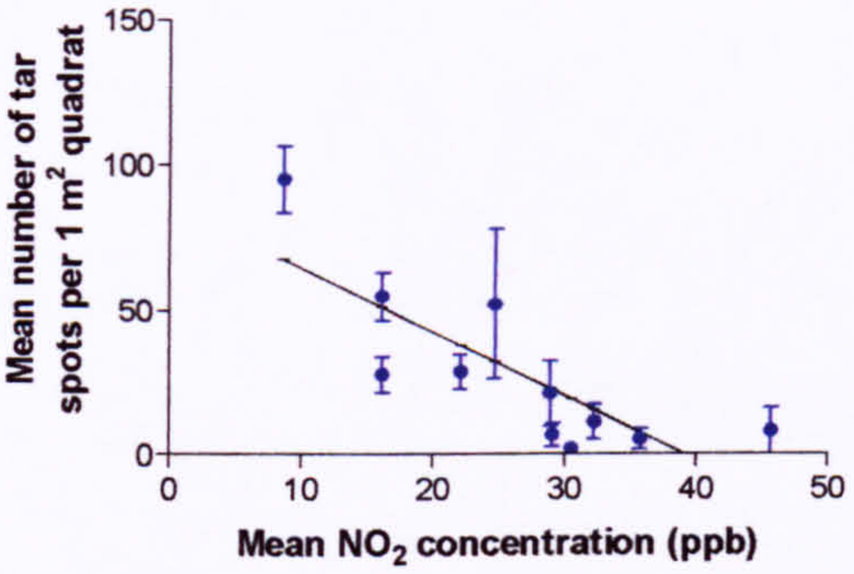


Figure 7.9 Numbers of overwintered tar spots (mean ± SE) in 1 m^2 quadrats in April 2002 plotted against mean NO_2 concentration (Nov 2001 – Sept 2002) for each site. $y = -2.2249x + 87.11$, $r^2 = 0.65$, $p = 0.0028$

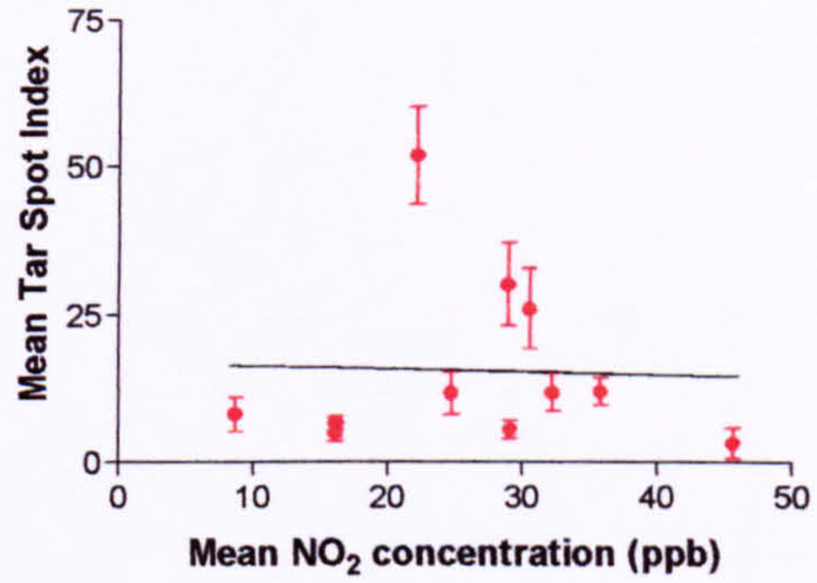


Figure 7.10 TSI for each site (mean ± SE) in Oct 2002 plotted against mean NO_2 concentration (Nov 2001 – Sept 2002) for each site. $y = -0.0506x + 17.101$, $r^2 = 0.0013$, $p = 0.92$

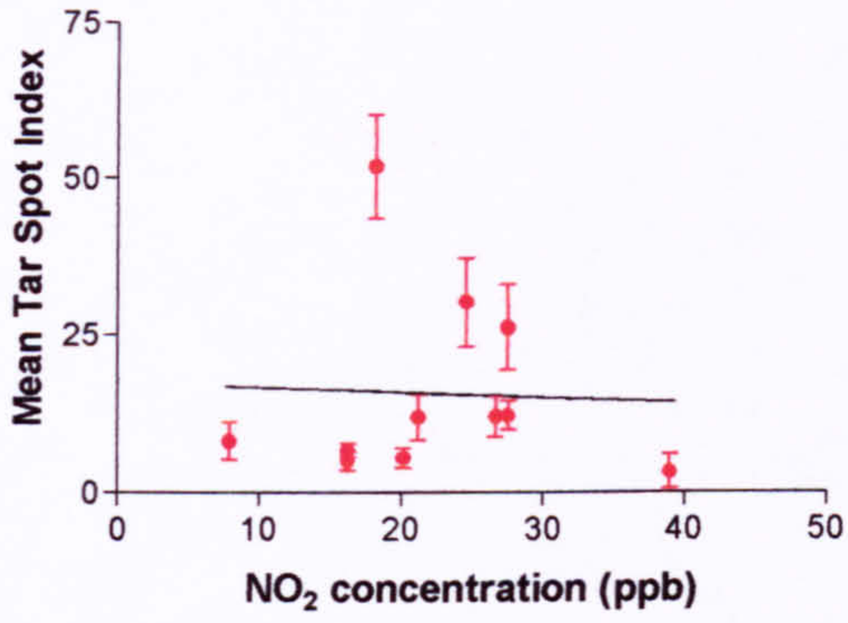


Figure 7.11 TSI for each site (mean ± SE) in Oct 2002 plotted against the NO_2 concentration in May 2002. $y = -0.0769x + 17.481$, $r^2 = 0.0018$, $p = 0.90$

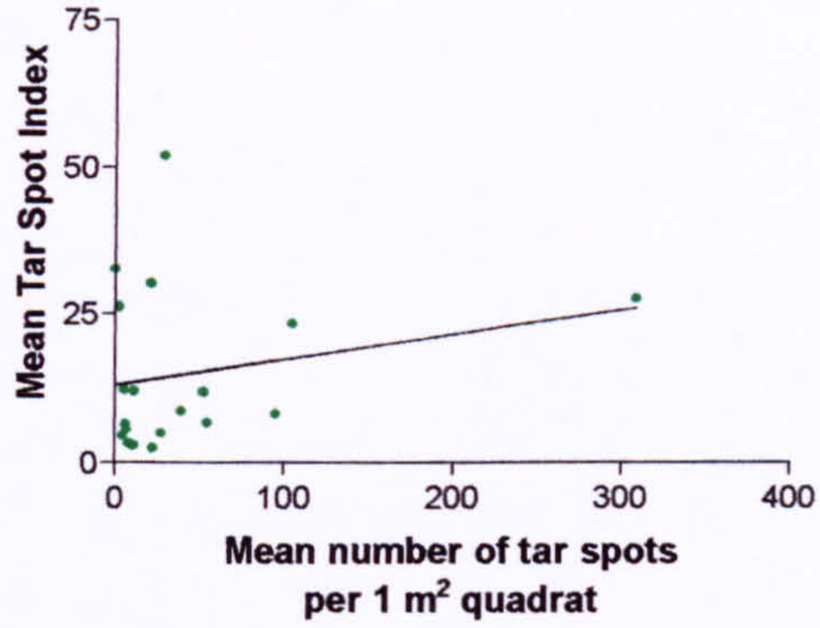


Figure 7.12 Mean TSI for each site plotted against mean numbers of overwintered tar spots for each site in 1 m^2 quadrats (2001 and 2002 data pooled). $y = 0.0419x + 13.086$, $r^2 = 0.049$, $p = 0.36$

7.4 Discussion

Urban pollution climates of several decades ago, dominated by SO_2, are known to have influenced the distribution of tar spot disease. The disease is still less widespread in urban compared with rural areas. It is possible that this represents a "memory effect", where the present-day distribution of a sensitive species is governed by past levels of a pollutant, for example due to a time-lag in re-colinisation. This has been found for certain SO_2-sensitive epiphytic lichens (e.g. Bates *et al.*, 1990). Re-invasion by tar spot disease would have been further hampered by the removal of inoculum-containing leaf litter at urban sites (Leith and Fowler, 1987). However, it is also possible that current pollution climates in urban areas are influencing the success of the disease.

Urban pollution mixtures inhibited tar spot infection in sycamore saplings supplied with inoculum in the Solardomes in spring 2002. Saplings in clean air treatments exhibited high tar spot indices, and a high proportion of leaves bore spots, while infection in polluted air Solardomes was negligible (Figures 7.4 and 7.5). The pollution treatment had no influence, however, on the development of tar spot disease. Sycamore saplings which had been recently infected before

being placed in the Solardome chambers in June 2000 showed no difference in infection rates between treatments in September 2000 (Figures 7.2 and 7.3). It can be concluded from these results that exhaust gas pollution inhibits tar spot disease, during the infection period only, at concentrations of 100 ppb NO_x. This is higher than the threshold found by Jarraud (2000).

Results from the field studies were complex. Although linear regression gave no significant relationship between TSI and NO_2 concentrations, we might not expect such a straightforward relationship. A number of local effects control the pattern of tar spot infection (Leith and Fowler, 1987), so that if NO_2 pollution does exert an effect, it might only dictate the maximum possible infection rate. If this were the case, then a plot of TSI against NO_2 concentration might be expected to give points covering a triangular area, with the widest part of the triangle at low NO_2 concentrations, becoming narrower as concentrations increase. The results given here could fit in with this pattern (Figures 7.8, 7.10 and 7.11), since TSIs at the most polluted sites are low in both years of study, but there are too few observations to give a clear picture.

The number of overwintered tar spots remaining at the base of the tree in spring (April 2002) showed a significant negative relationship with NO_2 concentrations (fig 8). However, if TSI itself is not negatively correlated with NO_2 at the concentrations found at these sites, then it is unlikely that there is a causal relationship between density of inoculum and pollution. It is more likely that the lack of inoculum in the more polluted areas is a result of its removal by wind and/or human activity in those areas, as suggested by Leith and Fowler (1987). The lack of correlation between density of inoculum and TSI (Figure 7.12) means that even when most of the leaf litter is removed, the small amount of inoculum remaining in spring must be enough to re-infect the trees. It is also possible that inoculum from outside the 4 m radius around the tree, where quadrats were placed, is available to cause infection of the trees.

This study has shown, under controlled environmental conditions, that urban pollution has the potential to inhibit tar spot disease. However, this inhibition only occurs at high pollution concentrations (e.g. 100 ppb total NO_x), which would only be found in highly polluted cities, such as London. It is not possible to conclusively assign this effect to NO_2 pollution without further fumigation studies. Another urban pollutant, or a combination of pollutants, could be the inhibitory agents. Field surveys in this study have shown that the distribution of infection by tar spot disease is complex, being influenced by many factors, so that any effect of pollution might be difficult to interpret.

Chapter 8: General Discussion

8. General Discussion

Plants growing in urban situations are subjected to a complex mixture of gaseous pollutants, as well as hostile environmental conditions such as low water availability coupled with elevated temperatures, air turbulence and compacted soils. The present study attempted to re-create a polluted roadside atmosphere under near-natural environmental conditions using a Solardome fumigation system, and looked at the effects of pollutant mixtures on trees and shrubs in combination with other factors commonly encountered in the urban environment. Initial screening experiments on 19 common urban species revealed interspecific variation in sensitivity to exhaust gas pollution.

Further study of a selection of species showed that urban pollution had only subtle effects on plant growth and biomass partitioning. It caused alterations in gas exchange in several species, which had the potential side effect of mitigating the damaging effects of drought stress. The presence of nitrogenous pollutants in the exhaust gas caused an up-regulation in the activity (in the leaves) of the enzyme nitrate reductase involved in nitrogen reduction, but did not have a marked effect on the tissue nitrogen content. The pollution accelerated the onset of senescence in some species, and had damaging effects on surface characteristics in *Quercus robur*. It impaired infection of sycamore by the fungal pathogen *Rhytisma acerinium*, the cause of tarspot disease.

8.1 The successes and limitations of the Solardome exposure system

The urban pollution Solardome exposure system was an attempt to create an approximation of an urban pollution climate under semi-controlled environmental conditions. Previous studies have also used fumigation systems in attempts to recreate roadside atmospheres. Sauter *et al.* (1987) and Viskari *et al.* (2000a; 2000b) carried out exposures of Norway spruce to exhaust gas mixtures. Sauter *et al.* (1987) used emissions from an Otto-motor at 560 ppm NO_x in a short (15 minute) fumigation. Viskari *et al.* (2000a; 2000b) fumigated plants in

controlled environment chambers with exhaust gas from a 4-stroke motor at 50, 100 and 200 ppb NO_x for 8 hours per day over 2- and 3-week periods. Sauter *et al.*'s (1987) study used extremely high concentrations of exhaust gases, which would not be encountered in real urban atmospheres. As with the work of Viskari *et al.* (2000a; 2000b), the present study exposed plants to concentrations of vehicle emissions that were close to those that occur in urban canyons. Here, this was performed under conditions that were as near-natural as possible. Unlike with previous studies, conditions of light and temperature were close to ambient. The Solardome chambers reduce irradiance in the PAR by 13-25% compared with ambient, depending on weather conditions. Temperature is elevated by between 2.4 °C on an overcast day to 5 °C on clear, sunny days when the external temperature is above 24 °C (Rafarel and Ashenden, 1991). The temperature increase might make conditions closer to that of a city, where the heat island effect increases the temperature by up to 6 °C (Chandler, 1965; Gilbert, 1989). This study also used longer exposure periods compared with previous work, which allowed plant responses to be tracked over a longer time.

The Solardome fumigation system had certain limitations. It did not re-create accurately the relative proportions of the different pollutants found in real urban pollution mixtures. All of the component gases of vehicle exhaust were represented, but were present in different ratios to those found in roadside atmospheres (Wellburn, 1990; Derwent, 1995; Reisinger, 2000; DoE, 1995). Due to the small scale of the experiment and the absence of uv radiation, there was no opportunity for the formation of the important secondary pollutant O_3 through photochemical reactions, and the associated changes in concentrations of O_3 precursors (i.e. NO_x and VOCs). This is an important shortcoming of the exposure system, since the presence of O_3 in real urban situations, when conditions favor its formation, would be expected to alter plant responses. O_3 is a powerful oxidant, and one of the most damaging pollutants to which vegetation is exposed (Fuhrer *et al.*, 1997; Barnes and Wellburn, 1998).

HONO, however, did seem to be formed in the chambers as a secondary pollutant. Upon combustion of fuel, HONO is directly emitted at only 1% of the concentration of NO_x, whereas the atmosphere in the Solardomes reached a concentration of 5.4 ppb HONO compared with around 100 ppb NO_x. This is a falsely high concentration compared with urban atmospheres. The HONO probably formed on available surfaces, i.e. the ground and the walls of the Solardomes, through the reaction of NO_x with water.

Apart from having different proportions of pollutants compared with the real urban atmosphere, the Solardome exposure also did not follow the diurnal pattern in concentrations found in cities, with peaks corresponding with rush-hours, which might influence plant responses. It was technically difficult to create fluctuations in the exhaust gas concentrations. Also, since a group of researchers were using the system, it was necessary to agree on an exposure that could be used in each group's experiments.

There were changes in the relative proportions of exhaust gases between experimental seasons, due to a refinement in the system between 2000 and 2001, and because of the generator being replaced between 2001 and 2002. In 2000, the generator was less reliable and there were periods when the generator was not functioning. This was reflected in a low average NO_x concentration (Table 2.1). NO_x were present close to the target concentration of 100 ppb in 2001 and 2002 (Table 2.1), but the $NO:NO_2$ ratio differed quite markedly between the two year (1.44 in 2001 compared with 1.94 in 2002).

Concentrations of benzene increased from 4.6 ng l^{-1} to 7.28 ng l^{-1} between 2001 and 2002 (Table 2.1), and those of toluene increased dramatically (from an average of 0.86 ng l^{-1} in 2001 to 5.11 ng l^{-1} in 2002; Table 2.1). The alteration in relative pollutant concentrations due to changes in the fumigation system is a possible explanation for the observed changes in certain plant responses between

different experiments. This and other possible causes for different findings between experiments will be discussed in more detail later.

Overall, the physical environment in the Solardomes was considered to be close to that of an urban environment but that exposure to the pollutant mixtures was greater than normal. It was a severe test of the capacity of plants to cope with urban pollution mixtures.

8.2 Effects of exhaust gas pollution on plant growth, biomass partitioning and leaf senescence

The exhaust gas pollution mixture had no effect on growth, measured as plant height, in any of the species studied. Growth has been shown to alter in response to nitrogen oxides in several species. Changes in growth in both directions have been observed, with NO tending to be more inhibitory to growth compared with NO_2 (Wellburn, 1990). VOCs have also shown properties of altering plant growth. Ethylene and isoprene are both emitted in vehicle exhaust, and both have been found to alter growth and development in plants (e.g. Goeschal and Kays, 1975; Terry *et al.*, 1995).

The presence of exhaust gas pollution tended to decrease the R:S ratio compared with plants growing in CFA. This was true in both *Cornus sanguinea* and *Ligustrum ovalifolium* (2001 experiment), but in the same two species this effect was reversed under conditions of simulated high soil nitrogen deposition (2002 experiment). There is much evidence that the presence of air pollutants generally reduces resource allocation to the roots, bringing about a reduction in R:S (reviewed in Davison and Barnes, 2002). In previous studies, NO_2 alone appears to have little effect on R:S, but there may be interactive effects of the gas with other pollutants, for example SO_2 (Darrall, 1989; Davison and Barnes, 2002). In the present study, the reduction in R:S brought about by the pollution appeared to be a combination of reduced allocation to roots and increased allocation to the shoots.

Although there was no effect of the urban pollution on growth and quite subtle effects on biomass partitioning over the course of a summer season, the pollution might have the potential to cause changes over longer periods. In slow-growing perennial species, growth effects might only become apparent over several growing seasons. Marked growth responses, in contrasting directions, were found in several herbaceous species under the same pollution regime in the Solardomes. Two herbaceous species showed a greater than 25% reduction in above-ground biomass in exhaust gas-polluted air compared with clean air controls, and six species showed a greater than 25% increase in biomass in response to the pollution treatment (Ashenden *et al.*, 2002; Sarah Honour, personal communication). In the present study, the pollution caused premature senescence in both *Cornus sanguinea* and *Ligustrum ovalifolium*, representing a shortening of the growing season (Bielenberg *et al.*, 2001). This could also potentially affect growth and biomass allocation in subsequent years. Accelerated senescence, manifested as alterations in mesophyll ultrastructure, was also found by Viskari *et al.* (2000b) in Norway spruce trees fumigated with exhaust gas mixtures.

The agent(s) responsible for this premature senescence are not known, but VOCs in the pollution mixture are a good candidate. Upon exposure to a mixture of VOCs, *Lotus corniculatus* plants were found to undergo premature senescence in the form of an acceleration in seed pod production (Cape *et al.*, 2003). Ethylene is known to be particularly important in determining the timing of flower and leaf senescence (e.g. Porat and Halevy, 1993; Davison, 1974).

8.3 Effects of exhaust gas pollution on gas exchange and photosynthesis

The exhaust gas pollution generally had the effect of decreasing stomatal conductance. In three of 16 species screened for stomatal response in 2000, conductance was reduced in plants in exhaust gas-polluted air compared with CFA. In all the four species studied in 2002, conductance was suppressed under

conditions of exhaust gas pollution. In the *Hydrangea* varieties, this effect was reversed during the night, when conductance was higher in exhaust gas-polluted atmospheres compared with CFA. This enhanced nocturnal conductance under exhaust gas exposure was also observed in Norway spruce by Viskari *et al.* (2000b), and might indicate enhanced respiration in an effort to repair pollution-induced damage. The generally decreased rates of conductance could be a direct response to one or more of the pollutants in the exhaust gas mixture. CO_2 is elevated in urban air (e.g. Nemitz *et al.*, 2002; Day *et al.*, 2002), and is known to bring about a strong stomatal closure response in some plant species (Robinson *et al.*, 1998). Lower conductance should reduce the overall pollutant dose, as well as lowering transpiration and water use.

The stomatal response of *Cornus sanguinea* altered between the 2001 and 2002 experimental seasons. In 2002, plants in exhaust gas-polluted air had *higher* conductance compared with those in CFA. One possible explanation for this change in response is the alteration in pollutant concentrations between the two seasons. This highlights that responses might alter depending on the relative proportions of pollutants, which is known to shift with distance from the pollution source. For example, the NO:NO_2 ratio at the roadside is around 4.0, while in background urban air, this value is close to 1.0 (e.g. Wellburn, 1990; Nielsen *et al.*, 1995).

Another possibility is that the differences in timing of the onset of exposure to the pollutants may have interfered with plant responses to the pollution. Discrepancies in the timing of fumigation between years of study are given in Table 2.3. Also, different timing of measurements relative to the start of exposure make the experiments from each year difficult to compare directly (Table 2.4). This is especially true for physiological parameters where there was an obvious shift in response throughout the year (e.g. stomatal conductance).

8.4 The influence of exhaust gas pollution on surface characteristics

Urban pollutant mixtures, both in the Solardome fumigations and in the field caused marked erosion of epicuticular waxes of *Quercus robur*, reflected in increased wettability of the cuticle, and damage to wax ultrastructure. This result is in agreement with previous studies of the effects of several types of pollution (e.g. Trimble *et al.*, 1982; Günthardt-Goerg and Keller, 1986; Günthardt-Goerg, 1988; Barnes *et al.*, 1990; Barnes and Brown, 1990; Maňkovská *et al.*, 1998), including exposures of Norway spruce to exhaust emissions (Sauter *et al.*, 1987; Viskari *et al.*, 2000a). Alterations in leaf surface characteristics and wettability can have far-reaching implications for the plant. It can reduce the water-retaining capabilities of leaves, with obvious consequences for water loss (e.g. Riederer and Marksätdter, 1996). Compared with water-repellent leaves, wettable leaves are liable to greater deposition of gaseous and particulate pollution, and higher retention of deposited particles (e.g. Neinhuis and Barthlott, 1998). This effect is expected to exacerbate pollution-induced damage to the cuticle. Erosion of the cuticle and associated increases in wettability may also have the potential to encourage attack by diseases and pests. For example, many fungal pathogens require free moisture on the leaf surface for successful hyphal growth and penetration (Huttunen, 1984).

Pollution-induced structural degradation of waxes could be useful as a bioindicator of urban pollution. In the present study, oak would be the obvious choice as a species that reflects the level of pollution in the condition of its waxes.

8.5 The influence of exhaust gas pollution in conjunction with plant stress

8.5.1 Drought

The pollution regime did not exacerbate drought stress in any of the species studied. In one species, *Ligustrum ovalifolium*, it mitigated the effects of drought, with exhaust gas-polluted plants wilting less rapidly compared with plants in CFA. Similar effects on drought avoidance properties have been found in

response to O_3, mediated by the effect of the pollutant in lowering stomatal conductance (reviewed in Mills, 2002). The effect of the pollution on wilting found here may have represented only a short-term protection against drought stress, however, since droughted plants in polluted air had lower R:S ratios compared with well-watered plants in CFA. Such a shift in biomass partitioning could impair the plants' future drought avoidance capabilities. This effect has also been observed in Timothy grass exposed to combinations of SO_2 and NO_2 (Lucas, 1990) and in soybean exposed to O_3 (Heggestad *et al.*, 1985).

8.5.2 Fungal pathogens

The intensity of infection by biotrophic plant pathogens has been shown to be generally reduced by air pollutants, mainly in studies of the effects of SO_2 and O_3 (reviewed in Bell *et al.*, 1993). This has also been found for *Rhytisma acerinium* infection of sycamore in the present study under urban polluted atmospheres at 100 ppb NO_x. This study confirms the results of field surveys and controlled field inoculations made in London by Jarraud (2000), where infection rates were found to increase along an urban-to-rural transect. The present study has also confirmed that it is during the infection period that the pollution influences the disease. It did not, however identify which component(s) of the exhaust gas mixture brought about the effects on the disease, or in finding the threshold concentration for pollution effects. Further study is required to address these questions.

8.5.3 Nitrogen deposition

Surprisingly, there were no marked interactions of exhaust gas pollution with simulated enhanced nitrogen deposition. But since nitrogen deposition represents prolonged exposure to nitrogenous pollutants, the responses of plants to extra soil nitrogen addition are of interest. The main effect of enhanced nitrogen addition was to decrease assimilation of nitrogen, reflected by reduced activity of NR, in the leaves of *Cornus sanguinea* and in the roots of *Ligustrum ovalifolium*. Other studies have also found that some tree species (e.g. spruce and beech)

decrease their uptake rates of NO_3^- and NH_4^+ when subjected to high loads of nitrogen, partially counteracting the effects of nitrogen deposition (Näsholm, 1998). In *Cornus sanguinea*, enhanced uptake of nitrogen did not lead to greater nitrogen concentrations in the leaves. *Ligustrum ovalifolium* leaves did contain more nitrogen in response to exhaust gas pollution, but only at high levels of simulated soil nitrogen deposition. The addition of nitrogen to the soil had a contrasting influence on NR activity compared with the presence of nitrogen compounds in the atmosphere as NO_x, which led to an increase in the activity of this enzyme in the leaves. Previous studies have also shown that the presence of atmospheric NO_2 often increases the activity of NR (e.g. Mansfield and Lucas, 1996; von Ballmoos *et al.*, 1998).

8.6 Identification of species useful for urban planting

Ligustrum ovalifolium has been planted for over a century in urban gardens and is well known for its tolerance to SO_2-dominated urban atmospheres of the past. The current work shows that it is also tolerant of current urban pollution climates.

Stomatal conductance in this species was generally suppressed under exhaust gas exposure, which would have the side effect of decreasing pollutant entry into the plant and therefore the effective pollutant dose. It was found to be highly resistant to drought compared with the other study species, which would be an advantage in the urban environment where plants have often to contend with conditions of low water supply. Moreover, its drought resistance capabilities were enhanced under exhaust gas exposure, at least in the short-term. There were no adverse effects on growth, although leaf abscission was brought on prematurely in plants growing in polluted atmospheres. Normally an evergreen species, *Ligustrum ovalifolium* has the added benefit of acting as a potential sink for pollutants throughout the year. This might however have long-term consequences for the plant since harmful contaminants could be accumulated over several seasons (Becket *et al.*, 1998).

The other main species studied here were not previously known to be tolerant, as none have such a history of urban planting as *Ligustrum ovalifolium*. It was expected therefore that one or more of these species might not be tolerant of exhaust gas pollution. However, it appears that all are fairly well suited to the urban situation.

8.7 Suggestions for future study

- More studies on the effects of pollutant mixtures on plants are required. The effects on plants of complex combinations of pollutant gases cannot be extrapolated from traditional exposures using single gases, or combinations of two or three gases (Barnes and Wellburn, 1998). Exposures should be of environmentally relevant concentrations, and should be carried out over long time periods, since it is also impossible to predict plant responses in the long-term from the results of short-term experiments.

- An open-air fumigation system might give more realistic conditions than have so far been achieved in exposure of plants to urban pollutants. This would allow plant responses to be studied under natural environmental conditions, and would not preclude the formation of secondary pollutants such as O_3 as with the system used in this study. Open-air systems have been successfully developed for exposure of plants to SO_2 (e.g. McLeod, 1995) and O_3 (e.g. Kainulainen *et al.*, 2000; Oksanen, 2003).

- Although further studies into relevant mixtures of pollutants are required, it would be of interest to identify which component(s) of the pollution mixture used here caused certain effects. For example, it is assumed that VOCs were responsible for damage to cuticles, but it is not known which VOC species would

be most reactive with cuticular components. The causal agent that deterred infection of sycamore by tar spot disease has also not been identified.

- It is possible that restriction of the root system by the pots could alter plant responses, for example in allocation of resources between roots and shoots. Ideally, plants should be grown in soil, without pots. This would entail a considerable increase in the scale of fumigation experiments.

- With *Rhytisma acerinium* (tar spot) it would be interesting to examine spore germination and hyphal growth, perhaps on an artificial medium. This could be performed under conditions of exhaust gas exposure, to ascertain at which point the pollution has its effect.

- This work was a preliminary investigation, using only one urban pollution regime. In order to contribute to the determination of critical levels for urban air pollutants, it would be instructive to expose plants to a range of concentrations of exhaust gas emissions. This would allow the identification of threshold concentrations for effects on different plant species.

- The mechanisms of control of NR, and how this relates to nitrogen metabolism needs further work. It would also be of interest to look at enzymes involved in later steps of nitrogen assimilation, and how these respond to exhaust gas pollution.

References

References

A

Aerts, R and Chapin, F. S. (2000) The mineral nutrition of wild plants: a re-evaluation of processes and patterns. Advances in Ecological Research 30: 1-67.

Alfiani, A., Maisto, G., Prati, M. V. and Baldantoni, D. (2001) Leaves of *Quercus ilex* L. As biomonitors of PAHs in the air of Naples (Italy). Atmospheric Environment 35: 3553-3559

Almbauer, R. A., Piringer, M., Baumann, K., Oettl, D. and Sturum, P. J. (2000) Analysis of the daily variations of wintertime air pollution concentrations in the city of Graz, Austria. Environmental Monitoring and Assessment 65: 79-87.

Anderson, L. S. and Mansfield, T. A. (1979) The effects of nitric oxide pollution on the growth of tomato. Environmental Pollution 20: 113-121.

Ashenden, T. W., Ashmore, M., Bell, J. N. B., Bignal, K., Binnie, J., Cape, J. N., Caporn, S. .J. N., Carroll, J., Davison, A. W., Hadfield, P., Honour, S., Lawton, K., Moore, S., Power, S. And Shields, C. (2002) Impacts of vehicle emissions on vegetation. Proceedings of the Eighth International Conference on Urban Transport and the Environment in the 21st Century held at Seville, Spain.

Atkins, D. H. F., Healy, C. and Tarrant, J. B. (1978) The use of simple diffusion tubes for the measurement of nitrogen dioxide levels in homes using gas and electricity for cooking. United Kingdom Atomic Energy Authority Report, AERE-R9 184

B

Bačić, T., Lynch, A. H. and Cutler, D. (1999) Reactions to cement factory dust contamination by Pinus halepensis needles. Environmental and Experimental Botany 41: 155-166.

Barnes, R. L. (1972) Effects of chronic exposure to ozone on photosyntesis and respiration of pines. Environmental Pollution 3: 133-138.

Barnes, J. D. and Brown, K. A. (1990) The inflence of ozone and acid mist on the amount and wettability of the surface waxes on needles of Norway spruce [*Picea abies* (L.) Karst.]. New Phytologist 114: 531-535.

Barnes, J. D., Davison, A. W. and Booth, T. A. (1988) Ozone accelerates structural degradation of epicuticular wax on Norway spruce needles. New Phytologist 110: 309-318.

Barnes, J. D., Eamus, D., Davison, A. W., Ro-Poulsen, H. and Mortensen, L. (1990) Persistent effects of oxone on needle water loss and wettability in Norway spruce. Environmental Pollution 63: 345-363.

Barnes, J. D. and Wellburn, A. R. (1998) Air pollutant combinations. In: Responses of plant metabolism to air pollution and global change. Eds: L. J. De Kok and I. Stulen. Backhuys Publishers, Leiden, The Netherlands.

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I. and Wilhelmi, H. (1998) Classification and terminology of plant epicuticular waxes. Journal of the Linnaen Society of London. Botany 126: 137-260.

Bates, J. W., Bell, J. N. B. and Farmer, A. M. (1990) Epiphyte recolinisation of oaks along a gradient of air pollution in south-east England. Environmental Pollution 68: 81-99.

Beckett, K. P., Freer-Smith, P. H. and Taylor, G. (1998) Urban woodlands: their role in reducing the effects of particulate pollution. Environmental Pollution 99: 347-360.

Bell, S., Ashenden, T. W. and Rafarel, C. R. (1992) Effects of rural roadside levels of nitrogen dioxide on polytrichum formosum Hedw. Environmental Pollution 76: 11-14.

Bell, J. N. B., McNeill, S., Houlden, G., Brown, V. S. and Mansfield, P. J. (1993) Atmospheric change: effects on plant pests and diseases. Parasitology, 106: 811-824

Bevan, R. J. and Greenhalgh, G. N. (1976) *Rhytisma acerinium* as a biological indicator of pollution. Environmental Pollution 10: 271-285

Bielenberg, D. G., Lynch, J. P. and Pell, E. J. (2001) A decline in nitrogen availability affects plant responses to ozone. New Phytologist 151: 413-425.

Bilger, H. W., Schreiber, U. and Lange, O. L. (1984) Determination of leaf heat-resistance - comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia 63: 256-262.

Binnie, J., Cape, J. N., Mackie, N. and Leith, I. D. (2002) Exchange of inorganic solvents between the atmosphere and grass – the use of open top chambers. The Science of the Total Environment 285:53-67

Björkman, O. and Demmig, B. (1987) Photon yield of O_2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. Planta 170: 489-504.

Black, C. R. and Black, V. J. (1979) The effects of low concentrations of sulphur dioxide on stomatal conductance and epidermal cell survival in field bean (*Vicia faba* L.). Journal of Experimental Botany 30: 291-298.

Black, V. J. and Unsworth, M. H. (1979) Effects of low concentrations of sulphur dioxide on net photosynthesis and dark respiration of *Vicia faba*. Journal of Experimental Botany 30: 473-483.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W. and Van den Wyngaert, I. J. J. (2002) Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. Background document for the Expert Workshop on Empirical Critical Loads for Nitrogen on (Semi-)natural Ecosystems. UN/ECE Convention on Long-range Transboundary Air Pollution. Berne, Swizerland.

Bobbink, R. and Lamers, L. P. M. (2002) Effects of increased nitrogen deposition. In: Air Pollution and Plant Life.

Bolsinger, M. and Flückiger, W. (1989) Ambient air pollution induced changes in amino acid pattern of phloem sap in host plants – relevance to aphid infestation. Environmental Pollution 56: 209-216.

Brouwer, R. (1962) Distribution of dry matter in the plant. Netherlands Journal of Agricultural Sciences 10: 361-376.

Brown, R. H. A., Cape, J. N. and Farmer J. G. Chlorinated hydrocarbons in Scots pine needles in northern Britain. Chemosphere 38: 795-806.

Burkhardt, J., Peters, K. and crossley, A. (1995) The presence of structural surface waxes on conifer needles affects the pattern of dry deposition of fine particles. Journal of Experimental Botany 46: 823-831.

C

Cape, J. N. (1983) Contact angles of water droplets on needles of Scots pine (*Pinus sylvestris*) growing in polluted atmospheres. New Phytologist 93, 293-299.

Cape, J. N. (1994) Evaluation of pollutant critical levels from leaf surface characteristics. In: Air pollutants and the leaf cuticle (eds K. E Percy *et al.*) pp. 97–105. NATO ASI Series, Vol G36, Springer-Verlag, Berlin Heidelberg.

Cape, J. N. (2003) Effects of airborne volatile organic compounds on plants. Environmental Pollution 122: 145-157

Cape, J. N., Fowler, D. and Davison, A. W. (2003a) Ecological effects of SO_2, fluorides and minor air pollutants: recent trends and research needs. Environment International 29: 201-211.

Cape, J. N., Leith, I. D., Binnie, J., Content, J., Donkin, M., Skewes, M., Price, D. N., Brown, A. R. and Sharpe, A. D. (2003b) Effects of VOCs on herbaceous plants in an open-top chamber experiment. Environmental Pollution 124: 341-353.

Cape, J. N. and Percy, K. E. (1993) Environmental influences on the development of spruce needle cuticles. New Phytologist 125: 787-799.

Cape, J. N. and Percy, K. E. (1996) The interpretation of leaf-drying curves. Plant, Cell and Environment 19: 356-361.

Caporn, S. J. M., Ashenden, T. W. and Lee, J. A. (2000) The effect of exposure to NO_2 and SO_2 on frost hardiness in *Calluna vulgaris*. Environmental and Experimental Botany 43: 111-119.

Capron, T. M. and Mansfield, T. A. (1976) Inhibition of net photosynthesis in tomato in air polluted with NO and NO_2. Journal of Experimental Botany 27: 1181-1186

Caporn, S. J. M., Risager, M. and Lee, J. A. (1994) Effect of nitrogen supply on frost hardiness in *Calluna vulgaris* (L.) Hull. New Phytologist 128: 461-468

Chamberlain, A. C. and Little, P. (1981) Transport and capture of particles by vegetation. In: Plants and their atmospheric environment. Eds: J. Grace, G. Ford and P. G. Jarvis. Blackwell, London.

Chandler, T. J. (1965) The climate of London. Hutchinson, London.

Clark, A. J., Landolt, W., Bucher, J. B., Strasser, R. J. (2000a) How wind affects the photosynthetic performance of trees: quantified with chlorophyll *a* fluorescence and open-top chambers. Photosynthetica 38: 349-360.

Clark, A. J., Landolt, W., Bucher, J. B. and Strasser, R. J. (2000b) Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll *a* fluorescence performance index. Environmental Pollution 109: 501-507.

Collins, C. D., Bell, J. N. B. and Crews, C. (2000) Benzene accumulation in horticultural crops. Chemosphere 40: 109-114

Conroy, J. P., Smillie, R. M., Kuppers, M., Bevege, D. I. and Barlow, E. W. (1986) Chlorophyll a fluorescence and photosynthetic and growth responses of Pinus radiata to phosphorus deficiency, drought stress and high CO_2. Plant Physiology 81:423-429.

D

Darrall, N. M. (1989) The effect of air pollutants on physiological processes in plants. Plant, Cell and Environment 12: 1-30.

Davison, A. W. (1974) The effects of ethylene on plants. In: Some gaseous pollutants in the environment. Report of a seminar held at the Royal Society, Carlton House Terrace. Inter-research Council Committee on Pollution Research.

Davison, A. W. and Barnes, J. D. (2002) Air pollutant-abiotic stress interactions. In: Air Pollution and Plant Life, Second Edition. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons, Ltd.

Day, T. A., Gober, P., Xiong, F. S. S. and Wentz, E. A. (2002) Temporal patterns of near-surface CO_2 concentrations over contrasting vegetation types in Phoenix metropolitan area. Agricultural and Forest Meteorology 110: 229-245.

De Kimpe, C. R. and Morel J. L. (2000) Urban soil management: a growing concern. Soil Science 165: 31-40.

De Kok, L. J. (1990) Sulfur metabolism in plants exposed to atmospheric sulfur. In: Sulfur nutrition and sulfur assimilation in higher plants. Eds: H. Rennenberg, C. Brunold, L. J. De Kok and I. Stulen. The Hague, The Netherlands: SPB Academic Publishing.

Derwent, R. G. (1995) Sources, distribution and fates of vocs in the atmosphere. In: Issues in Environmental Science and Technology 4: Volatile Organic Compounds in the Atmosphere. Eds: R.E. Hester, R.M. Harrison. The royal society of chemistry, Cambridge.

DoE (1994a) Expert panel on air quality standards: carbon monoxide. HMSO, London.

DoE (1994b) Expert panel on air quality standards: ozone. HMSO, London.

DoE (1995) Expert panel on air quality standards: particulates. HMSO, London.

DoE (1996) Expert panel on air quality standards: nitrogen dioxide. HMSO, London.

Dohmen, G. P., McNeill, S. and Bell, J. N. B. (1984) Air pollution increases *Aphis fabae* pest potential. Nature 307: 52-53.

Duyzer, J. and Fowler, D. (1994) Modeling land atmosphere exchange of gaseous oxides of nitrogen in Europe. Tellus Series B – Chemical and Physical Meteorology 46: 353-372

E

Egerton-Warburton, L. M. and Allen, E. B. Shifts in arbuscular micorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10: 484-496.

Eigenbrode, S. D. and Espelie, K. E. (1995) Effects of plant epicuticular lipids on insect herbivores. Annual Review of Entomology 40: 171-194.

F

Fangmeier, A., Bender, J., Weigel, H. –J. and Jäger, H. –J. (2002) Effects of pollutant mixtures. In: Air pollution and plant life. 2nd Ed. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons Ltd, Chichester.

Farmer, A. (2002) Effects of particulates. In: Air Pollution and Plant Life, Second Edition. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons, Ltd.

Farquhar, G. D. and Richards, R. A. (1984) Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. Australian Journal of plant physiology 11: 539-552.

Febo, A., Perrino, C. and Allegrini, I. (1996) Measurement of nitrous acid in Milan, Italy, by DOAS and diffusion denuders. Atmospheric Environment 30: 3599-3609

Fessenden, J. E. and Ehleringer, J. R. (2002) Age-related variations in of ecosystem respiration across a coniferous forest chronosequence in the Pacific Northwest. Tree Physiology 22: 159-167.

Field, T. S., Lee, D. W. and Holbrook, N. M. (2001) Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiology 127: 566-574.

Fowler, D. (2002) Deposition and uptake by vegetation. In: Air Pollution and Plant Life, Second Edition. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons, Ltd.

Fowler, D., Cape, J. N., Coyle, M., Smith, R. I., Hjellbrekke, A. –G., Simpson, D., Derwent, R. G. and Johnson C. E. (1999) Modelling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystems. Environmental Pollution 100: 43-45.

Fracheboud Y., Haldimann P., Leipner J. and Stamp P. (1999) Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). Journal of Experimental Botany 50: 1533-1540.

Freer-Smith, P. H. and Broadmeadow, M. S. J. (1996) Urban woodland and the benefits for local air quality. Arboriculture Advisory and Information Service Research Note, Farnham.

Fuhrer, J., Skarby, J. and Ashmore, M. R. (1997) Critical levels for ozone effects on vegetation in Europe. Environmental Pollution 97: 91-106.

Furukawa, A., Yokoyama M., Ushijima, T. and Totsuka, T. (1984) The effects of NO_2 and/or O_3 on photosynthesis of sunflower leaves. In: Studies on the Effects of Air Pollutant Mixtures on Plants. Part 1. Research Report from the Institute of Environmental Studies. Ibaraki, Japan, No. 65.

G

George, E. and Seith, B. (1998) Long-term effects of a high nitrogen supply to soil on the growth and nutritional status of young Norway spruce trees. Environmental Pollution 102: 301-306.

Gerdol, R., Bragazza, L., Marchesini, R., Medici, A., Pedrini, P., Benedetti, S., Bovolenta, A. and Coppi, S. (2002) Use of moss for monitoring organic and inorganic air pollution in urban and rural sites in Northern Italy. Atmospheric Environment 36: 4069-4075.

Gilbert, O. L. (1989) The ecology of urban habitats. Chapman and Hall, London.
Goodwin, J. W. L., Salway, A. G., Murells, T. P., Dore, C. J., Passant, N. R. and Eggleston, H. S. (2000). UK emissions of air pollutants 1970 – 1998, AEA Technology.

Goeschl, J. D. and Kays, S. J. (1975) Concentration dependencies of some effects of ethylene on etiolated pea, peanut, bean and cotton seedlings. Plant Physiology 55: 670-677.

Gojon, A., Plassard, C. and Bussy, C. (1994) Root-shoot distribution of NO_3^- assimilation in herbaceous and woody species. In: A Whole Plant Perspective on Carbon-Nitrogen Interactions. Eds: J. Roy and E. Garnier. SPB Academic Publishing, The Hague.

Good, J. E. G. (1990) Air pollution and tree health in relation to aboriculture. In: Forestry Commission Bulletin 97: Research for practical aboroculture. HMSO, London.

Goodwin, J. W. L., Salway, A. G., Murrells, T. P., Passant, N. R. and Eggleston, H. S. (2000) UK emissions of air pollutants 1970-1998, AEA Technology, AEAT/R/EN/0270.

Gordon, C., Woodin, S. J., Alexander, I. J. and Mullins, C. E. (1999) Effects of incresed temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytologist 142: 243-258.

Grantz, D. A., Zhang, X. J., Massman, W. J., Delany, A. and Pederson, J. R. (1997) Ozone deposition to a cotton (*Gossypium hirsutum* L.) field: Stomatal and surface wetness effects during the California ozone deposition experiment. Agricultural and Forest Meteorology 85: 19-31.

Greenhalgh, G. N. and Bevan, R. J. (1978) Response of *Rhytisma acerinium* to air pollution. Transactions of the British Mycological Society 71: 491-523

Gregg, J. W., Jones, C. G. and Dawson, T. E. (2003) Urbanization effects on tree growth in the vicinity of New York city. Nature 424: 183-187.

Grennfelt, P. and Thörnelöf, E. (Eds) (1992) Critical loads for nitrogen – a workshop report. Report Nord. Nordic Council of Ministers, Copenhagen.

Grill, D. and Golop, P. (1983) SEM investigations of different dust depositions on the surface of coniferous needles and the effect on needle wax. Aquilo Series Botany 19: 225-261.

Gulz, P. G. and Boor, G. (1992) Seasonal variation in the epicuticular wax ultrastructures of *Quercus robur* leaves. Zeitschrift Fur Naturforschung C – A Journal of Biosciences 47: 800-806.

Gulz, P. G. and Muller, E. (1992) Seasonal variation in the composition of epicuticular waxes of *Quercus robur* leaves. Zeitschrift Fur Naturforschung C – A Journal of Biosciences 47: 800-806.

Günthardt-Goerg (1988) Site-dependent changes in epicuticular wax structure of spruce. In: Proceedings of the 14th International Meeting for Specialists in Air Pollution Effects on forest Ecosystems. Eds: J. B. Butcher and I. Butcher-Wallin. IUFRO Project Group, Interlaken, Switzerland.

Günthardt-Goerg, M. S. and Keller, T. (1986) *Picea abies*: Needle surface after fumigation with low concentrations of SO_2 or O_3. In: Direct effects of dry and wet deposition on forest ecosystems – in particular canopy interactions. Proceedings COST-CEC Workshop, Lökeburg.

H

Hanson, P. J. and Lindberg, S. E. (1991) Dry deposition of reactive nitrogen-compounds – a review of leaf, conopy and non-foliar measurements. Atmospheric Environment A, 25: 1615-1634.

Harris, G. W., Carter, W. P. L., Winer, A. M., Pitts, J. N., Platt, U. and Perner, D. (1982) Observations of nitrous acid in the Los Angeles atmosphere and implications for the predictions of ozone-precursor relationships. Environmental Science and Technology 16: 414-419.

Harrison, R. M. (1996) Air pollution: sources, concentrations and measurement. In: Pollution: causes, effects and control. 3rd edition. Edited by R. M. Harrison. The royal society of chemistry, Cambridge.

Hättenschwiler, S. and Körner, C. (1997) Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. Oecologia 113: 104-114.

Heagle, A. S. (1973) Interactions between air pollutants and plant parasites. Annual Review of Phytopathology 11: 365-388

Heagle A. S. and Philbeck, R. B. (1979). Exposure techniques. In: Methodology for the Assessment of Air Pollution Effects on Vegetation. Eds: W. W. Heck, S.V. Krupa, S.N. Linzon and E.R. Frederick, E. R. Air Pollution Control Association, Pittsburgh, PA.

Heath, R. L. (1980) Initial events in injury to plants by air pollutants. Annual Review of Plant Physiology. 31: 395-431.

Heggestad, H. E., Gish, T. J., Lee, E. H. and Douglass, L. W. (1985) interaction between soil moisture stress and ambient ozone on growth and yields of soybean. Phytopathology 75: 472-477.

Hiatt, M. H. (1999) Leaves as an indicator of exposure to airborne volatile organic compounds. Environmental Science and Technology 33: 4126-4133.

Hornung, M. A., Sutton, M. A. and Wilson, R. B. (Eds) (1995) Mapping and modelling of critical loads for nitrogen. Report of a workshop held at Grange-over-Sands, Cumbria, UK under the auspices of the UN/ECE Convention on Long-range Transboundary Air Pollution, Working Group for Effects. Institute of Terrestrial Ecology, Penicuik, UK.

Houlden, G., McNeil, S., Aminu-Kano, M. and Bell, J. N. B. (1990) Air pollution and agricultural aphid pests 1: fumigation experiments with SO_2 and NO_2. Environmental Pollution 67: 305-314.

Hűttl, R. E. (1990) nutrient supply and fertilizer experiments in view of N saturation. Plant and Soil 128: 45-58.

Huttunen, S. (1984) Interactions of disease and other stress factors with atmospheric pollution In: Air pollution and plant life. Ed: M. Treshow. John Wiley and Sons, New York.

Huttunen, S. (1994) Effects of air pollutants on epicuticular wax structure. In: Air pollutants and the leaf cuticle (eds K. E Percy *et al.*) pp. 81-96. NATO ASI Series, Vol G36, Springer-Verlag, Berlin Heidelberg.

I

Ichimura, K. and Suto, K. (1998) Role of ethylene in acceleration of flower senescence by filament wounding in *Portulaca* hybrid. Physiologia Plantarium 104: 603-607.

Innes, J. L. (1988) Forest decline in urban areas – a comment. The Science of the Total Environment 72: 227-233.

Isidrov Aleroso, I., Monteverde, C. A. and Estoque, M. A. (1992) Diurnal variations of air pollution over metropolitan Manila. Atmosfera 5: 241-275.

Ito, O., Okano, K. and Totsuka, T. (1984) Effects of NO_2 and O_3 alone or in combination on kidney bean plants. II. Amino acid pool size and composition. Research Report of the National Institute of Environmental Studies, Japan 66: 15-24.

J

Jagels, R. (1994) Leaf wettability as a measure of air pollution effects. In: Air pollutants and the leaf cuticle (eds K. E Percy *et al.*) pp. 97–105. NATO ASI Series, Vol G36, Springer-Verlag, Berlin Heidelberg.

Jarraud, N. S. (2000) The effects of ambient air pollution on leaf pathogens of rose and sycamore. PhD Thesis, Imperial College, London

Jetter, R., Riederer, M. Lendzian, K. J. (1996) The effects of dry O_3, SO_2 and NO_2 on reconstituted epicuticular wax tubules. New Phytologist 133: 207-216.

Juniper, B. E. (1960) Growth and development and effect of the environment on the ultrastructure of plant surfaces. Journal of the Linnean Society (botany) 56: 413.

K

Kahle, H. (1993) Response of roots of trees to heavy metals. Environmental and Experimental Botany 33: 99-119.

Kainulainen, P., Utriainen, J., Holopainen, J. K., Oksanen, J. and Holopainen, T. (2000) Influence of elevated ozone and limited nitrogen availability on conifer seedlings in an open-air fumigation system: effects on growth, nutrient content, mycorrhiza, needle ultrastructure, starch and secondary compounds. Global Change Biology 6: 345-355.

Kammerbauer, H., Ziegler-Jöns, A., Selinger, H., Römmelt, R., Knoppik, D. and Hock, B. (1987) Exposure of Norway spruce at the highway border: effects on gas exchange and growth. Experientia 43: 1124-1125.

Kerfourn, C. and Gerrec, J. P. (1992) Modifications in the alkane composition of cuticular waxes from spruce needles (*Picea abies*) and ivy leaves (*Hedera helix*) exposed to ozone fumigation and acid fog – comparisons with leaves from declining spruce trees. Canadian Journal of Botany 70: 861-869.

Kersteins, G. and Lendzian, K. L. (1989) Interactions between ozone and plant cuticles. I. Ozone deposition and permeability. New Phytologist 112: 13-19.

Kessler, C. and U. Platt, (1984) Nitrous acid in polluted air masses. In: Physico-Chemical Behaviour of Atmospheric Pollutants (Proc.), eds. B. Versino and G. Angeletti, D. Reidel, Dordrecht, 412.

Keymeulen, R. Schamp, N. and Vanlangenhove, H. (1993) Factors affecting airborne monocyclic aromatic hydrocarbon uptake by plants. Atmospheric Environment A 27: 175-180.

Kojima, M. and Lovei, M. (2001) World Bank technical paper no. 508: Urban air quality management. Coordinating transport, environment and energy policies in developing countries. The World Bank, Washington, D.C.

Krause, G. H. and Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annual Reviews of Plant Physiology and Plant Molecular Biology 42: 313 –349.

Kupčinskienė, E (2001) Annual variations of needle surface characteristics of *Pinus sylvestris* growing near the emission source. Water, Air and Soil Pollution 130: 923-928.

Kuylenstiema, J. C. I., Hicks, W. K., Cinderby, S. and Cambridge, H. (1998) Critical loads for nitrogen deposition and their exceedence at European scale. Environmental Pollution 102: 591-598.

L

Lange, O. L., Heber, U., Schulze, E. D. and Ziegler, H. (1989) Atmospheric pollutants and plant metabolism. In: Forest Decline and Air Pollution, A Study of Spruce (*Picea abies*) on Acid Soils. Springer, Berlin.

Lea, P. J. and Miflin, B. (1974) An alternative route for nitrogen assimilation in higher plants. Nature 251: 614-616.

Lee, J. A. and Caporn, S. J. M. (1998) Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. New Phytologist 139: 127-134.

Lee, Y. N. and Schwartz, S. E. (1981) Reaction kinetics of NO_2 with liquid water at low particle pressures. Journal of Physical Chemistry 85: 840-848.

Leith, I. D. and Fowler, D. (1987) Urban distribution of *Rhytisma acerinium* (Pers.) Fries (tar spot) on sycamore. New Phytologist 108: 175-181

Lendzian, K. J. and Kersteins, G. (1991) Sorption and transport properties of gases and vapors in plant cuticles. Reviews in Environmental Contamination and Toxicology 121: 65-128.

Leyton, L. and Juniper, B. E (1963) Cuticle structure and water relations of pine needles. Nature 198: 770-771.

Lovett, G. M. (1994) Atmospheric deposition of nutrients and pollutants in North America: an ecological perspective. Ecological Applications 4: 629-650.

Lovett, G. M., Traynor, M. M., Pouyat, R. V., Carreiro, M. M., Zhu, W. and Baxter, J. W. (2000) Atmospheric deposition to oak forests along an urban-rural gradient. Environmental Science and Technology 34: 4294-4300.

Lucas, P. W. (1990) The effects of prior exposure to sulphur dioxide and nitrogen dioxide on the water relations of Timothy grass (*Phleum pratense*) under drought conditions. Environmental Pollution 66: 117-138.

M

Madders, M. and Lawrence, M. I. G. (1981) The role of woodland in urban pollution control. Quarterly Journal of Forestry 76: 256-261.

MaierMaercker, U. and Koch, W. (1991) Experiments on the control capacity of stomata of *Picea abies* (L.) Karst. after fumigation with ozone and in environmentally damaged material. Plant Plant, Cell and Environment 14: 175-184.

Maňkovská, B., Percy, K. and Karnosky, F. D. (1998) Impact of ambient tropospheric O3, co2 and particulates on the epicuticular waxes of aspen clones differing in O3 tolerance. Ekológia (Bratislava) 18: 200-210.

Mansfield, T. A. (1998) Stomata and plant water relations: does air pollution create problems? Environmental Pollution 101: 1-11.

Mansfield, T. A. and Lucas, P. W. (1996) Effects of gaseous pollutants on crops and trees. In: Pollution: causes, effects and control. 3rd edition. Edited by R. M. Harrison. The Royal Society of Chemistry, Cambridge.

Mansfield, T.A., and Majernik, O. (1970) Can stomata play a part in protecting plants against air pollutants? Environmental Pollution 1: 149-154.

Matzner, E. and Murach, D. (1995) Soil changes induced by air pollutant deposition and their implication for forests in central Europe. Water, Air and Soil Pollution 85: 63-76.

McLeod, A. R. (1995) An open-air fumigation system for exposure of young forest trees to sulfur-dioxide and ozone. Plant, Cell and Environment 18: 215-225.

McPhearson, E. G., Nowak, D. J. and Rowntree, R. E. (1994) Chicago's urban forest ecosystem: Results of the Chicago urban forest climate project. USDA General Technical Report NE-186.

Mecklenburg, R. A., Tukey, H. B. and Morgan, J. V. (1966) A mechanism for the leaching of calcium from foliage. Plant Physiology 41: 610-613.

Mendoza-Dominguez, A., Wilkinson, J. G., Yang, Y. J. and Russel, A. G. (2000) Modeling and direct sensitivity analysis of biogenic emissions impacts on regional ozone formation in the Mexico-US border area. Journal of the Air and Waste Management Association 50: 21-31.

Mikkelsen, T. N. and HeideJorgensen, H. S. (1996) Acceleration of leaf senescence in *Fagus sylvatica* L. by low levels of troposperic ozone demonstrated by leaf coulour, chlorophyll fluorescence and chloroplast ultrastructure. Trees – Structure and Function 10: 145-156.

Miller, H. G. and Miller, J. D. (1988) Response to heavy nitrogen applications in fertilizer experiments in British forests. Environmental Pollution 54: 219-232.

Mills, G. (2002) Modifications of plant response by environment conditions. In: Air Pollution and Plant Life, Second Edition. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons, Ltd.

Moonen, P. C., Binnie, J., Cape, J. N. and Ashenden, T. W. (1999) Impacts of vehicle emissions on vegetation. Feasibility Study (URGENT 2), NERC.

Morawska, L., Jayaratne, E. R, Mengersen, K., Jamriska, M. and Thomas, S. (2002) Differences in airborne particle and gaseous concentrations in urban air between weekdays and weekends. Atmospheric Environment 36: 4375-4383.

Muller, B., Touraine, B. and Rennenberg, H. (1996) Interactions between atmospheric and pedosperic nitrogen nutrition in spruce (*Picea abies* L. Karst) seedlings. Plant, Cell and Environment 19: 345-355.

Murlis, J. (1995) VOCs: the development of UK policy. In: Issues in Environmental Science and Technology 4: Volatile Organic Compounds in the Atmosphere. Eds: R.E. Hester, R.M. Harrison. The royal society of chemistry, Cambridge.

Musselman, R. C., Minnick, T. J. (2000) Nocturnal stomatal conductance and ambient air quality standards for ozone. Atmospheric Environment 34: 719-733.

N

Nakji, T., Fukami, M., Dokiya, Y. and Izuta, T (2001) Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees – Structure and Function 15: 453-461.

Näsholm, T. (1998) Qualitative and quantitative changes in plant nitrogen acquisition induced by anthropogenic nitrogen deposition. New Phytologist 139: 87-90.

Näsholm, T., Edfast, A.B., Ericsson, A. and Norden, L. G. (1994) Accumulation of amino acids in some boreal forest plants in response to increased N availability. New Phytologist 126: 137-143.

Natori, T. and Totsuka, T. (1984) Effects of mixed gas on transpiration rate of several woody plants. I. Interspecific difference in the effects of mixed gas on transpiration rate. Research Report of the National Institute for Environmental Studies No. 15. Ibaraki, Japan.

NEGTAP (2001) Acidification, Eutrophication and Ground-Level Ozone in the UK. Report of the National Expert Group on Transboundary Air Pollution. DERFA, UK.

Neighbour, E. A., Cottam, D. A. and Mansfield, T. A. (1988) Effects of sulphur dioxide and nitrogen dioxide on the control of water loss by birch (*Betula* spp.) New Phytologist 108: 149-157.

Neinhuis, C. and Barthlott, W. (1998) Seasonal changes in leaf surface contamination in beech, oak and ginkgo in relation to leaf micromorphology and wettability. New Phytologist 138: 91-98.

Nemitz, E., Hargreaves, K. J., McDonald, A. G., Dorsey, J. R. and Fowler, D. (2002) Micrometeorological measurements of the urban heat budget and CO_2 emissions on a city scale. Environmental Science and Technology 36: 3139-3146.

Nielsen, T., Jørgensen, H. E., Grundahl, L., Jensen, A. B., Nielsen, P. A. and Tønnesen, J. (1995) Traffic PAH and other air pollutants in the center of a large city. Risø-R-787. Risø National Laboratory, Roskilde, Denmark.

Notholt, J., Hjorth, J. and Raes, F. (1992) Formation of HNO_2 on aerosol surfaces during foggy periods in the presence of NO and NO_2. Atmospheric Environment 26a: 211-217.

O

Ögren, E. (1990) Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. Plant Physiology 93: 1280-1285.

Ohlson, M., Nordin, A. and Näsholm, T (1995) Accumulation of amino acids in forest plants in relation to ecological amplitude and nitrogen supply. Functional Ecology 9: 596-605.

Oksanen, E. (2003) Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. Plant, Cell and Environment 26: 875-886.

Owen, S. M., MacKenzie, A. R., Stewart, H., Donovan, R. and Hewitt, C. N. (2003) Biogenic volatile organic compound (VOC) emission estimates from an urban tree canopy. Ecological Applications 13: 927-938.

P

Padgett, P. E. and Allen, E, B. (1999) Differential responses to nitrogen fertilization in native shrubs and exotic annuals common to Mediterranean coastal sage scrub of California. Plant Ecology 144: 93-101.

Pal, A., Kulshreshta, K., Ahmed, K. J. and Behl, H. M. (2002) Do leaf surface characters play a role in plant resistance to auto-exhaust pollution? Flora 197: 47-55.

Pearson, M. and Mansfield, T. A (1993) Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). New Phytologist 123: 351-358.

Percy, K. E. and Baker, E. A. (1990) Effects of simulated acid rain on epicuticular wax production, morphology, chemical composition and on cuticular membrane thickness in two clones of sitka spruce (*Picea sitchensis* Carr.). New Phytologist 116: 79-87.

Porat, R. and Halevy, A. H. (1993) Enhancement of petunia and dendrobium flower senescence by jasmonic acid methyl-ester is via the promotion of ethylene production. Plant Growth Regulation 13: 297-301.

PORG (1997) Ozone in the UK (4th report of the Photochemical Oxidants Review Group). Department of the Environment, Transport and Regions, London (ITE Edinburgh).

Port, G. R. and Thompson, J. R. (1980) Outbreaks of insect herbivores on plants along motorways in the United Kingdom. Journal of Applied Ecology 17: 649-656.

Prasad, B. J. and Rao, D. N. (1980) Alteration in metabolic pools of nitrogen dioxide exposed wheat plants. Indian Journal of Experimental Biology 18: 879-882.

R

Rabe, E. (1990) Stress physiology: the functional significance of the accumulation of nitrogen-containing compounds. Journal of Horticular Sciences 65: 231-243.

Rafarel, C. R. and Ashenden, T. W. (1991) A facility for the large-scale exposure of plants to gaseous atmospheric pollutants. New Phytologist 117: 345-349

Rafarel CR, Ashenden TW and Roberts TM (1995) An improved Solardome system for exposing plants to elevated CO2 and temperature. New Phytologist 131: 481-490.

Reid, D. M. and Watson, K. (1985) Ethylene as an air pollutant. In: Ethylene and plant development. Eds: J. A. Roberts and G. Tucker. Butterworths.

Reisinger, A. R. (2000) Observations of HNO_2 in the polluted winter atmosphere: possible heterogeneous production on aerosols. Atmospheric Environment 34: 3865-3874.

Ricks, G. R. and Williams, R. J. H. (1974) effects of atmospheric pollution on deciduous woodland part 2: effects of particulate matter upon stomatal diffusion resistance in leaves of *Quercus petraea* (Mattuschka) Leibl. Environmental Pollution 6: 87-109.

Riederer, M. and Markstädter, C. (1996) Cuticular waxes: a critical assessment of current knowledge. In: Plant cuticles: an integrated functional approach. Ed. G. Kersteins. Bios Scientific Publishers, Oxford.

Riederer, M. and Schneider, G. (1989) Comparitive study of the composition of waxes extracted from isolated leaf cuticles and from whole leaves of citrus – evidence for selective extraction. Physiologia Plantarium 77: 373-384.

Riemer, J. and Whittaker, J. B. (1989) Air pollution and insect herbivores: observed interactions and possible mechanisms. Insect Plant Interactions 1: 74-105.

Robinson, M. F., Heath, J. and Mansfield, T. A. (1998) Disturbances in stomatal behavior caused by air pollutants. Journal of Experimental Botany 49: 461-469.

Rose, J. (1994) Control strategies sought for nitrogen deposition. Environmental Science and Technology 28: 559.

Rowland, A., Drew, M. C. and Wellburn, A. (1987) Foliar entry and incorporation of tropospheric nitrogen dioxide into barley plants of different nitrogen status. New Phytologist 107: 357-371.

Rowland, A., Murray, A. J. S. and Wellburn, A. (1985) Oxides of nitrogen and their impact upon vegetation. Reviews on Environmental Health V: 295-345.

S

Saarinen, T. (1993) Chlorophyll fluorescence and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats. Annales Botanici Fennici 30: 1-7.

Salway, A. G., Eggleston, H. S., Goodwin, J. W. L. and Murrells, T. P. (1996) UK emissions of air pollutants, 1970-1996. National Atmospheric Emissions Inventories. DoE, UK.

Saunders, P. J. W. (1965) Studies of the blackspot disease of roses, caused by *Diplocarpon rosae*. PhD Thesis, Exeter University, UK.

Sauter, J. J., Kammerbauer, H., Pambor, L. and Hock, B. (1987) Evidence for the accelerated micromorphological degradation of epistomatal waxes in Norway spruce by motor vehicle emissions. European Journal of Forest Pathology 17: 444-448.

Sauter, J. J. and Pambor, L. (1989) The dramatic effect of road side exposure and of aromatic hydrocarbons on the epistomatal waxes in Norway spruce and its significance for Waldsterben. European Journal of Forest Pathology 19: 370-378.

Sauter, J. J. and Voss, J. –U. (1986) SEM observations on the structural degradation of epistomatal waxes in picea abies (L.) Karst. and its possible role in the "Fichtensterben". European Journal of Forest Pathology 16: 408-423.

Saxe, H. (1986) Effects of NO, NO_2 and CO_2 on net photosynthesis, dark respiration and transpiration of pot plants. New Phytologist 103: 185-197.

Schenone, G., Fumagalli, I., Mignanego, L., Montinaro, F. and Soldatini, G. F. (1994) Effects of ambient air pollution in open-top chambers on bean (*Phaseous vulgaris* L.). New Phytologist 126: 309-315.

Schreuder, M. D. J., van Hove, L. W. A. and Brewer, C. A. (2001) Ozone exposure affects leaf wettability and tree water balance. New Phytologist 152: 443-454.

Shan, Y., Feng, Z., Izutu, T., Aoki, M. and Totsuka, T. (1996) The individual and combined effect of ozone and simulated acid rain on growth, gas exchange and water use efficiency of *Pinus armandi* Franch. Environmental Pollution 91: 355-361.

Silva-Fernandes, A. M. S. (1983) Studies on plant cuticles VIII. Surface waxes in relation to water repellency. Annals of Applied Biology 56: 297-304.

Simpson, D., Winiwarter, W., Borjesson, G., Cinderby, S., Ferreriro, A., Guenther, A., Hewitt, C. N., Janson, R., Khalil, M. A. K., Owen, S., Pierce, T. E., Puxbaum, H., Shearer, M., Skiba, U., Steinbreacher, R., Tarrason, L., and Oquist, M. G. (1999) Inventorying emissions from nature in Europe. Journal of Geophysical Research 104: 8113-8152.

Spencer, H. J. and Port, G. R. (1988) Effects of roadside conditions on plants and insects. II. Soil conditions. Journal of Applied Ecology 25: 709-715.

Spencer, H. J., Scott, N. E, Port, G. R. and Davison, A. W. (1988) Effects of roadside conditions on plants and insects. I. Atmospheric conditions. Journal of Applied Ecology 25: 699-707.

Srivastava, H. S. and Ormrod, D. P. (1984) Effects of nitrogen dioxide and nitrate nutrition on growth and nitrogen assimilation in bean leaves. Plant Physiology 76: 418.

Strasser R. J., Srivasatava A. and Govindjee, A. (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria, Photochemistry and Photobiology 61: 32-34.

Stuhl, F. and Niki, H. (1972) Journal of Chemical Physics 57: 3671.

Stulen, I. and Ter Steege, M. (1995) Light and nitrogen assimilation. In: Nitrogen nutrition in higher plants. Eds: H. S. Srivastava, R. P. Singh. New Delhi, India. Associated Publishing.

Stulen, I., Perez-Soba, M., De Kok L. J. and Van Der Eerden, L. (1998) Impact of gaseous nitrogen deposition on plant functioning. New Phytologist 139: 61-70

T

Tamm, C. O. (1991) Denitrification in forest ecosystems – preface. Forest Ecology and Management 44: 1-3.

Temple, P. J., Benoit, L. F., Lennox, R. W., Reagan, C. A. and Taylor, O. C. (1988) Combined effects of ozone and water stress on alfalfa growth and yield. Journal of Environmental Quality 17: 108-113.

Terry, G. M., Stokes, N. J., Hewitt, C. N. and Mansfield, T. A. (1995) Exposure to isoprene promotes flowering in plants. Journal of Experimental Botany 46: 1629-1631.

Thomas, R. C. and Mead, D. J. (1992) Uptake of nitrogen by *Pinus radiata* and retention in the soil after applying 15N-labelled urea at different frequencies. I. Growth response and nitrogen budgets. Forest Ecology and Management 53: 131-151.

Thompson, J. R., Mueller, P. W., Flückiger, W. and Rutter, A. J. (1984) The effect of dust on photosynthesis and its significance for roadside plants. Environmental Pollution 34: 171-190.

Tingey, D. T. and Hogsett, W. E. (1985) Water stress reduces ozone injury via a stomatal mechanism. Plant Physiology 77: 944-947.

Treshow, M. and Bell, J. N. B. (2002) Historical Perspectives. In: Air pollution and plant life. Second Edition. Eds: J. N. B. Bell and M. Treshow. John Wiley and Sons Ltd, Chichester, UK.

Trimble, J. L., Skelly, J. M., Tolin, S. A. and Orcutt, D. M. (1982) Chemical and structural characterisation of the needle epicuticular wax of two clones of *Pinus strobus* differing in sensitivity to ozone. Phytopathology 72: 652-656.

Turunen, M. and Huttunen, S. (1990) A review of the response of epicuticular wax of conifer needles to air pollution. Journal of Environmental Quality 19: 35-45.

U

Unsworth, M. H. (1990) Air pollution and vegetation – hypothesis, field exposure and experiment. Proceedings of the Royal Society of Edinburgh (B) 97: 139-153.

USEPA website www.epa.gov/region5/air/mobile/auto_emis.htm.

V

Van Hove, L. W. A., Van Kooten, O., Van Wijk, K. J., Vredenberg, W. J., Adema, E. H. and Pieters, G. A. (1991) Physiological effects of a long term exposure to low concentrations of SO_2 and NH_3 on poplar leaves. Physiologia Plantarum 82: 31-40.

Viskari, E. L., Holopainen, T. and Kärenlampi, L. (2000b) Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions - II ultrastructural changes and stomatal behavior. Environmental Pollution 107: 99-107

Viskari, E. L., Kossi, S. and Holopainen, J. K. (2000c) Norway spruce and spruce shoot aphid as indicators of traffic pollution. Environmental Pollution 107: 305-314.

Viskari, E. L., Surakka, J., Pasanen, P., Mirme, A., Kossi, S., Ruuskanen, J. And Holopainen, J. K. (2000a) Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions – I plant-insect interactions. Environmental Pollution 107: 89-98.

von Balmoos, P., Ammann, M., Egger, A., Suter, M. and Brunold, C. (NO_2-induced nitrate reductase activity in needles of Norway spruce (*Picea abies*) under laboratory conditions. Physiologia Plantarum 102: 596-604.

W

Weathers, K. C., Cadenasso, M. L. and Pickett, T. A. (2001) Forest edges as nutrient and pollutant concentrators: potential synergisms between fragmentation, forest canopies, and the atmosphere. Conservation Biology 15: 1506-1514.

Wellburn, A. R. (1998) Atmospheric nitrogenous compounds and ozone – is NOx fixation by plants a possible solution? New Phytologist 139:5-9.

Wellburn, A. R. (1990) Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? New Phytologist 115: 395-429.

White, K. L., Hill, A. C. and Bennett, J. H. (1974) Synergistic inhibition of apparent photosynthesis rate of alfalfa by combinations of sulphur dioxide and nitrogen dioxide. Environmental Science and Technology 8: 574-576.

Whitehead, S. J., Caporn, S. J. M. and Press, M. C. (1997) effects of elevated CO_2, nitrogen and phosphorous on the growth and photosynthesis of two upland perennials: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytologist 135: 201-211.

Whittaker, J. B. (1994) Interactions between insects and air pollution. In: Plant responses to the gaseous environment. Eds: Alscher, R. G. and Wellburn, A. R. Chapman and Hall, London.

Winner, W. E. (1994) Mechanistic analysis of plant responses to air pollution. Ecological Applications 4: 651-661

Y

Yoneyama, T., Hashimoto, A. and Totsuka, T. (1980) Absorption of atmospheric NO_2 by plants and soils. IV. Two routes of nitrogen uptake by plants from atmospheric NO_2: direct incorporation into aerial plant parts and uptake by roots after absorption into the soil. Soil Science and Plant Nutrition 26: 1-7.

Yoneyama, T., Totsuka, T., Hashimoto, A. and Yazaki, J. (1979) Absorption of atmospheric NO_2 by plants and soils. III. Changes in the concentration of inorganic nitrogen in the soil fumigated with : the effect of water conditions. Soil Science and Plant Nutrition 25: 337-347.

Appendices

Chapter 3

Appendix 1 Two-factor repeated measures ANOVA table for stomatal conductance in *Acer pseudoplatanus*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	1023.4	1	1023.4	0.103	0.752
Error	179427.2	18	9968.2		
Within Subjects					
Time	178234.6	1	178234.6	17.533	0.01
Time * Pollution	19031.4	1	19031.4	1.872	0.188
Error	182986.5	18	10165.9		

Appendix 2 Two-factor repeated measures ANOVA table for stomatal conductance in *Buddleja davidii*. There is a significant difference between time periods but not between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	304451.3	1	304451.3	3.565	0.075
Error	1537192.1	18	85399.6		
Within Subjects					
Time	395130.1	1	395130.1	6.358	0.02
Time * Pollution	20840.4	1	20840.4	0.345	0.564
Error	1087769.5	18	60431.6		

Appendix 3 Two-factor repeated measures ANOVA table for stomatal conductance in *Cornus sanguinea*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	3856	1	3856	0.483	0.496
Error	143569.5	18	7976.1		
Within Subjects					
Time	156630.5	1	156630.5	29.633	<0.001
Time * Pollution	9824.1	1	9824.1	1.859	0.19
Error	95142	18	5285.7		

Appendix 4 Two-factor repeated measures ANOVA table for stomatal conductance in *Euonymus japonicus*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	240.4	1	240.4	0.053	0.821
Error	81713.7	18	4539.7		
Within Subjects					
Time	306048.9	1	306048.9	27.251	<0.001
Time * Pollution	3744.9	1	3744.9	0.333	0.571
Error	202151.7	18	11230.6		

Appendix 5 Two-factor repeated measures ANOVA table for stomatal conductance in *Fagus sylvatica*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	81626.9	1	81626.9	2.128	0.164
Error	613691.4	16	38355.7		
Within Subjects					
Time	60600.5	1	60600.5	9.114	0.008
Time * Pollution	16052.1	1	16052.1	2.414	0.140
Error	106385.2	16	6649.1		

Appendix 6 Two-factor repeated measures ANOVA table for stomatal conductance in *Fraxinus excelsior*. There is no significant difference between time periods, or between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	4914.3	1	4914.3	0.317	0.581
Error	263475.3	17	15498.5		
Within Subjects					
Time	8	1	8.014	0.001	0.973
Time * Pollution	572.8	1	572.8	0.082	0.778
Error	118471.4	17	6968.9		

Appendix 7 Two-factor repeated measures ANOVA table for stomatal conductance in *Hydrangea macrophylla* "Lacecap". There is no significant difference between time periods, but a significant difference was found between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	65102.6	1	65102.6	10.420	0.005
Error	112461.1	18	6247.8		
Within Subjects					
Time	33304.4	1	33304.4	3.356	0.084
Time * Pollution	5857	1	5857	0.590	0.452
Error	178629.4	18	9923.9		

Appendix 8 Two-factor repeated measures ANOVA table for stomatal conductance in *Hydrangea macrophylla* "Pink". There is no significant difference between time periods, or between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	24462.2	1	24462.2	3.130	0.094
Error	140696.7	18	7816.5		
Within Subjects					
Time	2646	1	2646	0.840	0.372
Time * Pollution	2377.6	1	2377.6	0.755	0.396
Error	56721	18	3151.2		

Appendix 9 Two-factor repeated measures ANOVA table for stomatal conductance in *Hypericum androsaemum*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	36240.8	1	36240.8	1.62	0.219
Error	402645.9	18	22369.2		
Within Subjects					
Time	56419.9	1	56419.9	5.291	0.034
Time * Pollution	9504.6	1	9504.6	0.891	0.358
Error	191929.6	18	10662.8		

Appendix 10 Two-factor repeated measures ANOVA table for stomatal conductance in *Ligustrum ovalifolium*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	40664.1	1	40664.1	2.727	0.116
Error	268374.5	18	14909.7		
Within Subjects					
Time	203282.2	1	203282.2	9.478	0.006
Time * Pollution	5594.6	1	5594.6	0.258	0.617
Error	389866.5	18	21659.2		

Appendix 11 Two-factor repeated measures ANOVA table for stomatal conductance in *Pittosporum tenuifolium*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	2640.1	1	2640.1	0.099	0.757
Error	480397.7	18	26688.8		
Within Subjects					
Time	498405	1	498405	18.303	<0.001
Time * Pollution	6052.2	1	6052.2	0.222	0.643
Error	490158.7	18	27231		

Appendix 12 Two-factor repeated measures ANOVA table for stomatal conductance in *Rosa rubiginosa*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	13290.8	1	13290.8	0.225	0.641
Error	1063419.1	18	59078.8		
Within Subjects					
Time	778372.3	1	778372.3	31.248	<0.001
Time * Pollution	26271.6	1	26271.6	1.055	0.318
Error	448373.4	18	24909.6		

Appendix 13 Two-factor repeated measures ANOVA table for stomatal conductance in *Salix caprea*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	259284	1	259284	1.968	0.180
Error	2108174.6	16	131760.9		
Within Subjects					
Time	318886.4	1	318886.4	11.630	0.004
Time * Pollution	57998.1	1	57998.1	2.115	0.165
Error	438697.2	16	27418.6		

Appendix 14 Two-factor repeated measures ANOVA table for stomatal conductance in *Sambucus nigra*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	105504.3	1	105504.3	0.725	0.406
Error	2618509.1	18	145472.7		
Within Subjects					
Time	410434.6	1	410434.6	7.147	0.016
Time * Pollution	52293	1	52293	0.911	0.353
Error	1033705.6	18	57428.1		

Appendix 15 Two-factor repeated measures ANOVA table for stomatal conductance in *Sorbus aria*. There is a significant difference between time periods and between pollution treatments, plus a significant interaction between pollution treatment and time periods.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	18012.8	1	18012.8	6.146	0.023
Error	52757.5	18	2930.9		
Within Subjects					
Time	85514.2	1	85514.2	12.435	0.020
Time * Pollution	23931.7	1	23931.7	3.480	0.078
Error	123780.9	18	6876.7		

Appendix 16 Two-factor repeated measures ANOVA table for stomatal conductance in *Viburnum davidii*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	13984.3	1	13984.3	1.040	0.321
Error	242098.7	18	13449.9		
Within Subjects					
Time	267665	1	267665	23.670	<0.001
Time * Pollution	47713	1	47713	4.219	0.055
Error	205345.9	18	11308.1		

Appendix 17 Two-factor repeated measure ANOVA table for RWC in *Cornus sanguinea*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.05	1	0.05	0.945	0.344
Error	0.96	18	0.05		
Within Subjects					
Time	11.6	1	11.6	318.255	<0.001
Time * Pollution	0.09	1	0.09	2.391	0.139
Error	0.66	18	0.036		

Appendix 18 Two-factor repeated measure ANOVA table for RWC in *Hebe carnosula*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.00046	1	0.00046	0.002	0.969
Error	0.522	18	0.029		
Within Subjects					
Time	2.4	1	2.4	75.814	<0.001
Time * Pollution	0.0076	1	0.0076	0.242	0.629
Error	0.565	18	0.031		

Appendix 19 Two-factor repeated measure ANOVA table for RWC in *Hydrangea macrophylla* "Pink". There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.0093	1	0.0093	1.078	0.313
Error	0.156	18	0.0086		
Within Subjects					
Time	1.974	1	1.974	116.008	<0.001
Time * Pollution	0.0053	1	0.0053	0.311	0.584
Error	0.306	18	0.017		

Appendix 20 Two-factor repeated measure ANOVA table for RWC in *Pittosporum tenuifolium*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.015	1	0.015	2.245	0.151
Error	0.123	18	0.0068		
Within Subjects					
Time	0.567	1	0.567	116.323	<0.001
Time * Pollution	0.0088	1	0.0088	1.811	0.195
Error	0.087	18	0.0049		

Appendix 21 Two-factor repeated measure ANOVA table for RWC in *Rosa rubiginosa*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.0018	1	0.0018	0.024	0.879
Error	1.342	18	0.074		
Within Subjects					
Time	11.5	1	11.5	243.664	<0.001
Time * Pollution	0.0051	1	0.0051	0.108	0.746
Error	0.852	18	4.7		

Appendix 22 Two-factor repeated measure ANOVA table for RWC in *Salix caprea*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.055	1	0.055	0.407	0.532
Error	2.5	18	0.14		
Within Subjects					
Time	21.6	1	21.6	108.818	<0.001
Time * Pollution	0.029	1	0.029	0.150	0.703
Error	3.6	18	3.6		

Appendix 23 Two-factor repeated measure ANOVA table for RWC in *Viburnum davidii*. There is a significant difference between time periods, but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.0034	1	0.0034	1.644	0.216
Error	0.038	18	0.0021		
Within Subjects					
Time	0.471	1	0.471	282.070	<0.001
Time * Pollution	0.011	1	0.011	6.667	0.019
Error	0.03	18	0.0017		

Chapter 4

Appendix 24 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Cornus sanguinea*. There is a significant difference between time periods and between pollution treatments, plus a significant interaction between pollution treatment and time periods.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	22363.5	1	22363.5	6.610	0.022
Error	47369.4	14	3383.5		
Within Subjects					
Time	576455.5	1	576455.5	114.714	<0.001
Time * Pollution	52206.5	1	52206.5	10.389	0.006
Error	70351.9	14	5025.1		

Appendix 25 Two-factor repeated measures ANOVA table for stomatal conductance on 25th August 2001 in *Cornus sanguinea*. There is a significant difference between time periods and between pollution treatments, plus a significant interaction between pollution treatment and time periods.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	245965.1	1	245965.1	18.569	0.001
Error					
Within Subjects					
Time	517497.4	1	517497.4	30.622	<0.001
Time * Pollution	101505.2	1	101505.2	6.006	0.028
Error	236592	14	16899.4		

Appendix 26 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Ligustrum ovalifolium*. There is a significant difference between time periods and between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	26912	1	26912	4.358	0.050
Error					
Within Subjects					
Time	546411.8	1	546411.8	21.739	<0.001
Time * Pollution	40428	1	40428	1.608	0.225
Error	351892.9	14	25135.2		

Appendix 27 Two-factor repeated measures ANOVA table for stomatal conductance on 25th August 2001 in *Ligustrum ovalifolium*. There is a significant difference between time periods but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	15487.4	1	15487.4	1.568	0.231
Error	138316.893	14	9879.8		
Within Subjects					
Time	191940.7	1	191940.7	26.202	<0.001
Time * Pollution	16888.9	1	16888.9	2.306	0.151
Error	102556.5	14	7325.5		

Appendix 28 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Hydrangea macrophylla* "Pink" during the day. There is a significant difference between time periods but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	58158.1	1	58158.1	3.843	0.070
Error	212373.6	14	15169.5		
Within Subjects					
Time	242723.6	1	242723.6	13.268	0.003
Time * Pollution	19375.8	1	19375.8	1.059	0.321
Error	256112.5	14	18293.8		

Appendix 29 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Hydrangea macrophylla* "Pink" during the night. There is no significant difference between time periods but a significant difference was found between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	10484.3	1	10484.3	9.797	0.007
Error	14982.7	14	1070.2		
Within Subjects					
Time	3932.8	1	3932.8	4.311	0.057
Time * Pollution	102.6	1	102.6	0.113	0.742
Error	12771.6	14	912.3		

Appendix 30 Two-factor repeated measures ANOVA table for stomatal conductance on 25th August 2001 in *Hydrangea macrophylla* "Pink". There is a significant difference between time periods but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	2377.3	1	2377.3	0.075	0.788
Error	445070.6	14	31790.8		
Within Subjects					
Time	646888.5	1	646888.5	29.184	<0.001
Time * Pollution	13495.6	1	13495.6	0.609	0.448
Error	310323.9	14	22166		

Appendix 31 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Hydrangea macrophylla* "Lacecap" during the day. There is a significant difference between time periods but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	9169.9	1	9169.9	1.402	0.256
Error	91598.2	14	6542.7		
Within Subjects					
Time	50854.1	1	50854.1	5.189	0.039
Time * Pollution	5535.9	1	5535.9	0.565	0.465
Error	137211.5	14	9800.8		

Appendix 32 Two-factor repeated measures ANOVA table for stomatal conductance on 27th June 2001 in *Hydrangea macrophylla* "Lacecap" during the night. There is a significant difference between time periods and between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	4250.7	1	4250.7	60.252	<0.001
Error	987.7	14	70.5		
Within Subjects					
Time	386.6	1	386.6	4.825	0.045
Time * Pollution	75.4	1	75.4	0.941	0.348
Error	1121.6	14	80.1		

Appendix 33 Two-factor repeated measures ANOVA table for stomatal conductance on 25th August 2001 in *Hydrangea macrophylla* "Lacecap". There is a significant difference between time periods but none between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	4.9	1	4.9	0.001	0.972
Error	54579.3	14	3898.5		
Within Subjects					
Time	225221.6	1	225221.6	36.581	<0.001
Time * Pollution	3876.1	1	3876.1	0.630	0.441
Error	86194.1	14	6156.7		

Appendix 34 Three-factor repeated measures ANOVA table for water potential in *Cornus sanguinea*. There is a significant difference between days, pollution treatments and drought treatments, plus a significant interaction between days and drought treatment.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	4.8	1	4.8	21.926	<0.001
Drought	5.9	1	5.9	26.833	<0.001
Pollution * Drought	0.038	1	0.038	0.172	0.684
Error	3.7	17	0.22		
Within Subjects					
Days	33.3	1	33.3	23.411	<0.001
Days * Pollution	1.1	1	1.1	0.800	0.384
Days * Drought	8.2	1	8.2	5.738	0.028
Days * Pollution * Drought	0.58	1	0.58	0.411	0.530
Error	24.1	17	1.4		

Appendix 35 Three-factor repeated measures ANOVA table for soil moisture in *Cornus sanguinea*. There is a significant difference between days and drought treatments, plus a significant interaction between days and drought treatment.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.062	1	0.062	3.531	0.075
Drought	1.9	1	1.9	112.682	<0.001
Pollution * Drought	0.0046	1	0.0046	0.261	0.615
Error	0.351	20	0.018		
Within Subjects					
Days	1.2	1	1.2	59.319	<0.001
Days * Pollution	0.039	1	0.039	2.028	0.170
Days * Drought	0.4	1	0.4	20.415	<0.001
Days * Pollution * Drought	0.028	1	0.028	1.425	0.246
Error	0.39	20	0.019		

Appendix 36 Three-factor repeated measures ANOVA table for water potential in *Ligustrum ovalifolium*. There is a significant difference between days, pollution treatments and drought treatments. There is a significant interaction between days and drought treatment, and between pollution treatments and drought treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	9.7	1	9.7	56.489	<0.001
Drought	13.9	1	13.9	81.296	<0.001
Pollution * Drought	1.2	1	1.2	7.103	0.015
Error	3.3	19	0.17		
Within Subjects					
Days	27.9	1	27.9	31.250	<0.001
Days * Pollution	3.5	1	3.5	3.882	0.064
Days * Drought	21.2	1	21.2	23.738	<0.001
Days * Pollution * Drought	3.9	1	3.9	4.375	0.050
Error	16.9	19	0.89		

Appendix 37 Three-factor repeated measures ANOVA table for soil moisture in *Ligustrum ovalifolium*. There is a significant difference between days and drought treatments, plus a significant interaction between days and drought treatment.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.015	1	0.015	2.936	0.102
Drought	2.8	1	2.8	541.439	<0.001
Pollution * Drought	0.0096	1	0.0096	1.892	0.184
Error	0.10	20	0.0051		
Within Subjects					
Days	0.79	1	0.79	53.810	<0.001
Days * Pollution	0.0076	1	0.0076	0.512	0.482
Days * Drought	0.82	1	0.82	55.293	<0.001
Days * Pollution * Drought	0.019	1	0.019	1.274	0.272
Error	0.29	20	0.015		

Appendix 38 Three-factor repeated measures ANOVA table for water potential in *Hydrangea macrophylla* "Pink". There is a significant difference between days, pollution treatments and drought treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.23	1	0.23	5.906	0.029
Drought	0.47	1	0.47	12.380	0.003
Pollution * Drought	0.10	1	0.10	2.620	0.128
Error	0.54	14	0.038		
Within Subjects					
Days	3.5	1	3.5	13.556	0.002
Days * Pollution	0.33	1	0.33	1.296	0.274
Days * Drought	1.1	1	1.1	4.371	0.055
Days * Pollution * Drought	0.14	1	0.14	0.560	0.466
Error	3.6	14	0.26		

Appendix 39 Three-factor repeated measures ANOVA table for soil moisture in *Hydrangea macrophylla* "Pink". There is a significant difference between days and drought treatments, plus a significant interaction between days and drought treatment.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.0050	1	0.0050	0.965	0.338
Drought	0.49	1	0.49	95.915	<0.001
Pollution * Drought	0.00080	1	0.00080	0.155	0.698
Error	0.104	20	0.0052		
Within Subjects					
Days	0.44	1	0.44	43.118	<0.001
Days * Pollution	0.016	1	0.016	1.550	0.288
Days * Drought	0.34	1	0.34	38.756	<0.001
Days * Pollution * Drought	0.012	1	0.012	1.15	0.296
Error	0.206	20	0.010		

Appendix 40 Three-factor repeated measures ANOVA table for water potential in *Hydrangea macrophylla* "Lacecap". There is a significant difference between days.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.055	1	0.055	0.165	0.690
Drought	0.11	1	0.11	0.321	0.579
Pollution * Drought	0.033	1	0.033	0.098	0.758
Error	5.3	16	0.33		
Within Subjects					
Days	8.9	1	8.9	9.263	0.008
Days * Pollution	0.36	1	0.36	0.375	0.549
Days * Drought	1.9	1	1.9	1.966	0.180
Days * Pollution * Drought	1.1	1	1.1	1.083	0.314
Error	15.5	16	0.97		

Appendix 41 Three-factor repeated measures ANOVA table for soil moisture in *Hydrangea macrophylla* "Lacecap". There is a significant difference between days and drought treatments, plus a significant interaction between days and drought treatment.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	0.00018	1	0.00018	0.026	0.875
Drought	2.5	1	2.5	367.212	<0.001
Pollution * Drought	0.000009	1	0.000009	0.001	0.971
Error	0.14	20	0.0069		
Within Subjects					
Days	0.40	1	0.40	30.672	<0.001
Days * Pollution	0.0043	1	0.0043	0.331	0.572
Days * Drought	0.53	1	0.53	40.863	<0.001
Days * Pollution * Drought	0.0090	1	0.0090	0.692	0.415
Error	0.26	20	0.013		

Appendix 42 Effects of exhaust gas pollution and drought on carbon isotope discrimination ($\Delta^{13}C$) in *Ligustrum ovalifolium* grown in CFA, CFA under imposed drought, exhaust gas pollution and exhaust gas pollution under imposed drought. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and drought were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

$\Delta^{13}C$				Main effects					
CFA	Drought	Exhaust gas	Exhaust gas + Drought	Drought		Exhaust gas		Exhaust gas * Drought	
				F	P	F	P	F	P
21.60 ±0.61	19.05 ±0.66	18.53 ±0.77	18.32 ±0.35	3.21	n.s.	6.98	0.021	2.13	n.s.

Appendix 43 Three-factor repeated measures ANOVA table for plant height in *Cornus sanguinea*. There is a significant difference between days but none between pollution treatments or drought treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	85.6	1	85.6	0.613	0.439
Drought	107.5	1	107.5	0.770	0.386
Pollution * Drought	1.2	1	1.2	0.009	0.927
Error	4890.5	35	139.7		
Within Subjects					
Days	16148.1	1	16148.1	102.441	<0.001
Days * Pollution	75.7	1	75.7	0.480	0.493
Days * Drought	189.4	1	189.4	1.201	0.281
Days * Pollution * Drought	12.5	1	12.5	0.079	0.780
Error	5517.1	35	157.6		

Appendix 44 Three-factor repeated measures ANOVA table for plant height in *Ligustrum ovalifolium*. There is a significant difference between days but none between pollution treatments or drought treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	134.5	1	134.5	0.953	0.335
Drought	6.8	1	6.8	0.049	0.827
Pollution * Drought	35.3	1	35.3	0.250	0.620
Error	5080.3	36	141.119		
Within Subjects					
Days	70062.4	1	70062.4	377.890	<0.001
Days * Pollution	132.8	1	132.8	0.717	0.403
Days * Drought	123.6	1	123.6	0.667	0.420
Days * Pollution * Drought	50.7	1	50.7	0.273	0.604
Error	6674.6	36	185.4		

Appendix 45 Three-factor repeated measures ANOVA table for plant height in *Hydrangea macrophylla* "Pink". There is a significant difference between days but none between pollution treatments or drought treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	13.5	1	13.5	0.600	0.443
Drought	0.16	1	0.16	0.007	0.933
Pollution * Drought	26.1	1	26.1	1.159	0.289
Error	831.9	37	22.5		
Within Subjects					
Days	13604.9	1	13604.9	398.4	<0.001
Days * Pollution	40.5	1	40.5	1.185	0.283
Days * Drought	27.0	1	27.0	0.791	0.380
Days * Pollution * Drought	52.8	1	52.8	1.547	0.221
Error	1263.6	37	34.2		

Appendix 46 Three-factor repeated measures ANOVA table for plant height in *Hydrangea macrophylla* "Lacecap". There is a significant difference between days and between pollution treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	359.9	1	359.9	6.425	0.016
Drought	16.9	1	16.9	0.302	0.586
Pollution * Drought	15.8	1	15.8	0.282	0.599
Error	1960.7	1	56.0		
Within Subjects					
Days	9105.7	1	9105.7	93.553	<0.001
Days * Pollution	126.6	1	126.6	1.300	0.262
Days * Drought	79.2	1	79.2	0.814	0.373
Days * Pollution * Drought	97.8	1	97.8	1.005	0.323
Error	3406.6	35	97.3		

Appendix 47 Effects of exhaust gas pollution and drought on dry weights of plant parts in *Cornus sanguinea* grown in CFA, CFA under imposed drought, exhaust gas pollution and exhaust gas pollution under imposed drought. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and drought were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Dry weight (g)				Main effects					
	CFA	Drought	Exhaust gas	Exhaust gas + Drought	Drought		Exhaust gas		Exhaust gas * Drought	
					F	P	F	P	F	P
Roots	59.32 ±7.70	51.27 ±8.19	42.46 ±4.33	54.20 ±5.17	0.08	n.s.	1.13	n.s.	2.28	n.s.
Woody stems	15.26 ±1.74	13.13 ±0.89	16.74 ±1.69	17.61 ±0.97	0.21	n.s.	4.65	0.043	1.18	n.s.
Leaves	40.31 ±2.25	31.87 ±2.42	42.27 ±1.37	42.04 ±2.22	4.24	n.s.	8.31	0.009	3.80	n.s.
Above ground	55.57 ±2.85	44.99 ±2.95	59.01 ±2.64	59.66 ±3.08	2.96	n.s.	9.84	0.005	3.78	n.s.
Root : Shoot	1.05 ±1.10	1.12 ±0.14	0.71 ±0.05	0.89 ±0.05	1.94	n.s.	9.29	0.006	0.36	n.s.

Appendix 48 Effects of exhaust gas pollution and drought on dry weights of plant parts in *Ligustrum ovalifolium* grown in CFA, CFA under imposed drought, exhaust gas pollution and exhaust gas pollution under imposed drought. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and drought were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Dry weight (g)				Main effects					
	CFA	Drought	Exhaust gas	Exhaust gas + Drought	Drought		Exhaust gas		Exhaust gas * Drought	
					F	P	F	P	F	P
Roots	51.32 ±5.79	53.94 ±4.81	52.37 ±4.41	45.38 ±4.37	0.20	n.s.	0.59	n.s.	0.97	n.s
Woody stems	29.20 ±2.35	36.32 ±2.34	34.13 ±2.09	32.99 ±3.90	1.16	n.s.	0.08	n.s.	2.23	n.s.
Leaves	17.81 ±1.24	19.54 ±1.05	20.87 ±0.39	21.89 ±1.69	1.35	n.s.	3.16	n.s.	n.s.	n.s.
Above ground	47.01 ±3.46	55.86 ±3.14	54.99 ±1.90	54.87 ±5.56	1.35	n.s.	0.87	n.s.	0.87	n.s.
Root : Shoot	1.09 ±0.08	0.96 ±0.03	0.95 ±0.06	0.83 ±0.04	4.12	n.s.	4.42	0.048	0.01	n.s.

Chapter 5

Appendix 49 Three-factor repeated measures ANOVA Appendix for stomatal conductance in *Cornus sanguinea*. There is a significant difference between time periods but none between pollution treatments or nitrogen treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	132374.7	1	132374.7	3.199	0.081
Nitrogen	171484.2	3	57161.4	1.382	0.262
Pollution * Nitrogen	16408.3	3	5469.4	0.132	0.940
Error	1654989.1	40	41374.7		
Within Subjects					
Time	134066.5	1	134066.5	11.823	0.001
Time * Pollution	16790.2	1	16790.2	1.481	0.234
Time * Nitrogen	30775.5	3	10258.5	0.905	0.447
Time * Pollution * Nitrogen	14950.5	3	4983.5	0.439	0.726
Error	453578.6	40	11339.5		

Appendix 50 Three-factor repeated measures ANOVA Appendix for stomatal conductance in *Ligustrum ovalifolium*.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	7717.6	1	7717.6	1.738	0.195
Nitrogen	13211.8	3	4403.9	0.992	0.406
Pollution * Nitrogen	41316.0	3	13772.0	3.102	0.037
Error	177588.9	40	4439.7		
Within Subjects					
Time	1647.4	1	1647.4	1.071	0.307
Time * Pollution	3494.5	1	3494.5	2.271	0.140
Time * Nitrogen	14451.5	3	4817.163	3.131	0.036
Time * Pollution * Nitrogen	7278.4	3	2426.1	1.577	0.210
Error	61540.7	40	1538.5		

Appendix 51 Effects of exhaust gas pollution and nitrogen deposition on Fv/Fm and AREA values in *Ligustrum ovalifolium* grown in CFA, exhaust ga pollution and simulated nitrogen deposition at 10, 20 and 50 Kg N ha^{-1} y^{-1}. Values represent mean ±SE (n=8). Main and interactive effects of exhaust ga pollution and nitrogen deposition were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant differenc at the 5% level.

	Fv/Fm and AREA								Main effects					
	CFA	CFA + 10 Kg N ha^{-1} y^{-1}	CFA + 20 Kg N ha^{-1} y^{-1}	CFA + 50 Kg N ha^{-1} y^{-1}	Exhaust gas	Exhaust gas + 10 Kg N ha^{-1} y^{-1}	Exhaust gas + 20 Kg N ha^{-1} y^{-1}	Exhaust gas + 50 Kg N ha^{-1} y^{-1}	Nitrogen		Exhaust gas		Exhaust gas * Nitrogen	
									F	P	F	P	F	P
Fv/Fm	0.79 ±0.01	0.79 ±0.01	0.77 ±0.02	0.76 ±0.01	0.78 ±0.02	0.79 ±0.01	0.81 ±0.01	0.81 ±0.01	0.13	n.s.	5.65	0.021	1.71	n.s.
AREA	28625.00 ±2209.86	31325.00 ±2525.00	26514.23.00 ±1681.19	25300.00 ±1732.05	34514.29 ±3492.54	33450.00 ±2046.86	38025.00 ±2242.27	38050.00 ±2932.76	0.07	n.s.	22.81	<0.001	1.99	n.s.

Appendix 52 Effects of exhaust gas pollution and nitrogen deposition on nitrate reductase activity in *Cornus sanguinea* grown in CFA, exhaust gas pollution and simulated nitrogen deposition at 10, 20 and 50 Kg N ha^{-1} y^{-1}. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and nitrogen deposition were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Nitrate reductase activity								Main effects					
	CFA	CFA + 10 Kg N ha^{-1} y^{-1}	CFA + 20 Kg N ha^{-1} y^{-1}	CFA + 50 Kg N ha^{-1} y^{-1}	Exhaust gas	Exhaust gas + 10 Kg N ha^{-1} y^{-1}	Exhaust gas + 20 Kg N ha^{-1} y^{-1}	Exhaust gas + 50 Kg N ha^{-1} y^{-1}	Nitrogen		Exhaust gas		Exhaust gas * Nitrogen	
									F	P	F	P	F	P
Leaves	1.89 ±0.36	1.11 ±0.16	1.18 ±0.15	1.06 ±0.18	2.17 ±0.28	1.96 ±0.31	1.42 ±0.17	1.88 ±0.27	3.19	0.033	9.97	0.003	0.92	n.s.
Roots	1.06 ±0.16	0.89 ±0.13	0.94 ±0.18	1.10 ±0.16	0.91 ±0.15	1.19 ±0.20	0.96 ±0.08	0.94 ±0.12	0.51	n.s.	0.002	n.s.	1.84	n.s.

Appendix 53 Effects of exhaust gas pollution and nitrogen deposition on nitrate reductase activity in *Ligustrum ovalifolium* grown in CFA, exhaust gas pollution and simulated nitrogen deposition at 10, 20 and 50 Kg N ha^{-1} y^{-1}. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and nitrogen deposition were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Nitrate reductase activity								Main effects					
	CFA	CFA + 10 Kg N ha^{-1} y^{-1}	CFA + 20 Kg N ha^{-1} y	CFA + 50 Kg N ha^{-1} y	Exhaust gas	Exhaust gas + 10 Kg N ha^{-1} y^{-1}	Exhaust gas + 20 Kg N ha^{-1} y^{-1}	Exhaust gas + 50 Kg N ha^{-1} y^{-1}	Nitrogen		Exhaust gas		Exhaust gas * Nitrogen	
									F	P	F	P	F	P
Leaves	1.39 ±0.28	0.97 ±0.18	1.61 ±0.32	1.40 ±0.10	2.79 ±0.11	1.94 ±0.37	3.23 ±0.29	2.80 ±0.28	2.12	n.s.	20.72	<0.001	0.85	n.s.
Roots	0.50 ±0.07	0.37 ±0 02	0.24 ±0.03	0.27 ±0.03	0.47 ±0.02	0.28 ±0.02	0.19 ±0.03	0.22 ±0.02	26.81	<0.001	4.97	0.035	0.31	n.s.

Appendix 54 Three-factor repeated measures ANOVA Appendix for plant height in *Cornus sanguinea*. There is a significant difference between days but none between pollution treatments or nitrogen treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	482.1	1	482.1	2.578	0.114
Nitrogen	43.2	3	14.4	0.077	0.972
Pollution * Nitrogen	478.0	3	159.3	0.852	0.471
Error	10661.5	57	187.0		
Within Subjects					
Days	13603.1	1	13603.1	249.9	0.000
Days * Pollution	266.8	1	266.8	4.902	0.031
Days * Nitrogen	95.8	3	31.9	0.587	0.626
Days * Pollution * Nitrogen	74.8	3	24.9	0.458	0.713
Error	3101.9	57	54.4		

Appendix 55 Three-factor repeated measures ANOVA Appendix for plant height in *Ligustrum ovalifolium*. There is a significant difference between days but none between pollution treatments or nitrogen treatments.

Source	SS	DF	MS	F	P
Between Subjects					
Pollution	139.8	1	139.8	1.578	0.214
Nitrogen	243.6	3	81.2	0.916	0.439
Pollution * Nitrogen	216.2	3	72.1	0.814	0.492
Error	4961.4	56	88.6		
Within Subjects					
Days	3510.6	1	3510.6	138.529	0.000
Days * Pollution	105.5	1	105.5	4.164	0.046
Days * Nitrogen	105.9	3	35.3	1.393	0.254
Days * Pollution * Nitrogen	82.6	3	27.5	1.086	0.363
Error	1419.1	56	25.3		

Appendix 56 Effects of exhaust gas pollution and nitrogen deposition on shoot dry weight in *Cornus sanguinea* grown in CFA, exhaust gas pollution and simulated nitrogen deposition at 10, 20 and 50 Kg N ha^{-1} y^{-1}. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and nitrogen deposition were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

Shoot dry weight								Main effects					
CFA	CFA + 10 Kg N ha^{-1} y^{-1}	CFA + 20 Kg N ha^{-1} y	CFA + 50 Kg N ha^{-1} y	Exhaust gas	Exhaust gas + 10 Kg N ha^{-1} y^{-1}	Exhaust gas + 20 Kg N ha^{-1} y^{-1}	Exhaust gas + 50 Kg N ha^{-1} y^{-1}	Nitrogen		Exhaust gas		Exhaust gas * Nitrogen	
								F	P	F	P	F	P
11.63	14.03	14.98	18.93	12.57	16.40	14.60	22.10	7.77	0.001	1.59	n.s.	0.41	n.s.
±0.68	±1.26	±2.47	±1.68	±0.50	±2.08	±2.03	±1.18						

Appendix 57 Effects of exhaust gas pollution and nitrogen deposition on number of leaves remaining at the end of the season in *Ligustrum ovalifolium* grown in CFA, exhaust gas pollution and simulated nitrogen deposition at 10, 20 and 50 Kg N ha^{-1} y^{-1}. Values represent mean ±SE (n=8). Main and interactive effects of exhaust gas pollution and nitrogen deposition were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

Number of leaves								Main effects					
CFA	CFA + 10 Kg N ha^{-1} y^{-1}	CFA + 20 Kg N ha^{-1} y	CFA + 50 Kg N ha^{-1} y	Exhaust gas	Exhaust gas + 10 Kg N ha^{-1} y^{-1}	Exhaust gas + 20 Kg N ha^{-1} y^{-1}	Exhaust gas + 50 Kg N ha^{-1} y^{-1}	Nitrogen		Exhaust gas		Exhaust gas * Nitrogen	
								F	P	F	P	F	P
285.25	332.75	369.50	337.25	177.00	145.25	141.75	160.00	0.29	n.s.	97.22	<0.001	1.59	n.s.
±18.67	±32.65	±44.53	±44.14	±17.26	±12.52	±17.61	±9.41						

Appendix 58 Comparison of numbers of *Cornus sanguinea* leaves having different percentage coverage of red pigment in clean air and exhaust gas-polluted air. Comparison of treatments is by χ^2 with 9 d.f. Expected values are given in brackets. $\chi^2 = 38.186$; $p<0.001$ **

% of leaf covered by red pigment	Clean air	Exhaust gas-polluted air	Total
0-10	80 (65.87)	59 (73.13)	139
10-20	54 (44.54)	40 (49.46)	94
20-30	9 (11.85)	16 (13.15)	25
30-40	19 (23.22)	30 (25.78)	49
40-50	16 (20.85)	28 (23.15)	44
50-60	16 (17.53)	21 (19.47)	37
60-70	3 (9.48)	17 (10.52)	20
70-80	2 (10.9)	21 (12.10)	23
80-90	28 (24.17)	23 (26.83)	51
90-100	54 (52.60)	57 (58.40)	111
Total	281	312	593

Appendix 59 Effects of exhaust gas pollution and leaf age on Fv/Fm and AREA values in *Cornus sanguinea* grown in CFA and exhaust gas polluted air. Values represent mean ±SE (n=6). Main and interactive effects of exhaust gas pollution and leaf age were tested by twoway ANOVA. The level of significance is indicated by the p-value. n.s denotes no significant difference at the 5% level.

	Fv/Fm and AREA								Main effects					
	CFA (leaf 1)	CFA (leaf 2)	CFA (leaf 3)	CFA (leaf 4)	Exhaust gas (leaf 1)	Exhaust gas (leaf 2)	Exhaust gas (leaf 3)	Exhaust gas (leaf 4)	Leaf age		Exhaust gas		Exhaust gas * Leaf age	
									F	P	F	P	F	P
Fv/Fm	0.75 ±0.01	0.76 ±0.01	0.73 ±0.03	0.70 ±0.04	0.77 ±0.01	0.74 ±0.02	0.69 ±0.05	0.64 ±0.08	2.39	n.s.	1.16	n.s.	0.46	n.s.
AREA	30085.71 ±3117.34	26575.00 ±2184.18	17350.00 ±2515.03	14350.00 ±2154.31	32000.00 ±5286.82	25257.14 ±5588.03	19828.57 ±5983.54	11200.00 ±3556.08	8.25	<0.001	0.01	n.s.	0.23	n.s.